

## Article

# Use of Electric Cell-Substrate Impedance Sensing to Assess the Cytotoxic Effect of CBD on Human Oral Epidermoid Carcinoma Cells

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**Abstract:** Cannabidiol (CBD) is an active natural compound that is extracted from *Cannabis sativa*. Previous studies show that CBD is a non-psychoactive compound with significant anti-cancer effects. This study determines its cytotoxic effect on oral cancer cells and OEC-M1 cells and compares the outcomes with a chemotherapeutic drug, cisplatin. This study has investigated the effect of CBD on the viability, apoptosis, morphology and migration of OEC-M1 cells. Electric cell-substrate impedance sensing (ECIS) is used to measure the change in cell impedance for cells that are treated with a series concentration of CBD for 24 hours. AlamarBlue and annexin V/7-AAD staining assays show that CBD has a cytotoxic effect on cell viability and induces cell apoptosis. ECIS analysis shows that CBD decreases the overall resistance and morphological parameters at 4 kHz in a concentration-dependent manner. There is a significant reduction in the wound-healing recovery rate for cells that are treated with 30  $\mu$ M CBD. This study demonstrates that ECIS can be used for in vitro screening of new chemotherapy and is more sensitive, functional and comprehensive than traditional biochemical assays. CBD also increases cytotoxicity on cell survival and the migration of oral cancer cells, so it may be a therapeutic drug for oral cancer

**Keywords:** CBD; electric cell-substrate impedance sensing; oral cancer.

## 1. Introduction

Taiwan has a substantially higher incidence of oral cancer than other countries in the world. The risk of oral cancer is increased by behaviors including smoking, consuming alcohol, and chewing betel nuts. [1, 2]. People who use cigarettes, alcohol, and betel nuts have a 123 times higher chance of acquiring oral cancer than those who do not, according to epidemiological research conducted in 1995 by Professor Ge Yingqin at Kaohsiung Medical University. [3, 4]. Consuming too many refined carbohydrates can lead to

persistent inflammation [5] and abnormal malignant cell growth, which can result in cancer invasion or metastasis to the distal area. Oropharyngeal and oral tissues can both develop oral cancer. Squamous carcinoma, verrucous carcinoma, salivary gland cancer, and malignant melanoma are examples of cancer cell types with regard to histology. Squamous cell carcinoma and epidermoid cells are the most prevalent cell types. [6, 7].

Oral cancer can frequently spread to the lymphatic system in the neck, jaw, chin, and upper and middle neck. It can also spread to the lymphoid tissue under the collarbone, the liver, the lung, and the bone. In addition to surgery to remove the cervical lymph node, surgical resection is utilized to treat mouth cancer. Postoperative chemotherapy or radiation is then administered. [8, 9]. Then, patients need to have thorough follow-up exams on a regular schedule. Alternative cancer treatments are necessary since metastatic oral cancer has a poor prognosis and places a heavy burden on society and the medical system. Chemotherapy can be given intravenously, topically, orally, or intraperitoneally. It is a systemic therapy that the circulatory system distributes throughout the entire body. In order to restrict the disease's progression, relieve its symptoms, and stop metastasis, it is frequently employed to eliminate any remaining cancer cells following surgery. Chemotherapy slows the development of cancer cells by interfering with the synthesis of DNA, RNA, and proteins. It also makes surgery and radiation therapy more effective by preventing the advancement of the cell cycle. Cisplatin was utilized in this study to simulate a standard therapeutic treatment.

Apoptosis, sometimes known as "programmed death," is a natural occurrence. [10]. Different with the necrosis, apoptosis does not induce inflammation. Apoptosis is a type of cell suicide and many of its pathways are identified [11]. Previous research used biochemical-based assay to characterize between malignant and normal phenotype before regulating the development or deaths of cancer cells to determine the reason of apoptosis in cancer cells [10, 12]. In this study, we are trying to exam the possibility of the Cannabidiol (CBD) as an anti-cancer drug.

Several natural compounds, such as CBD, are holding a promising effect of anti-cancer treatment. CBD is extracted from *Cannabis sativa*. The formula for the compound is  $C_{21}H_{30}O_2$ . CBD is a natural compound with a molecular weight of 314.47 and is one of the main active ingredients of *Cannabis sativa* [29]. CBD has anti-inflammatory and anti-ischemic effects and is used to treat neuropathic pain and has an anti-tumor effect. CBD and  $\Delta$ -9-tetrahydrocannabinol (THC), the two main substances found in cannabis plants, each have various pharmacological and therapeutic effects on the human body. CBD does not have any of the psychoactive effects of  $\Delta$ -9-tetrahydrocannabinol (THC), whereas THC has both psychoactive and addictive qualities [30-32]. Although the molecular mechanism underlying CBD's anti-cancer activity is not well explored, the bulk of research demonstrate that it prevents cancer cells from proliferating by inducing apoptotic signaling. [33, 34]. According to previous studies, CBD alters signaling pathways crucial to the growth and spread of cancer, preventing the continuation of the cell cycle and reducing cell migration. [35, 36]. Animal studies have shown that CBD is efficient at halting the development of malignancies. [30, 31]. Additionally, it is said to trigger apoptosis in cancer cells by activating caspase pathways. [37]. Currently, CBD is widely consumed in European countries and America as an extract or oil from cannabis plants. CBD may be useful in treating cancer because it causes a significant inhibition of tumor growth, angiogenesis and metastasis in various cancer models[38-40]. Therefore, CBD may be of use for cancer therapy. However, it is still unclear whether CBD exhibits anti-tumorigenic activity in oral squamous cell carcinoma cells (OSCCs). This study determines the mechanisms that cause CBD-induced cell death in OSCCs and determines the synergistic efficacy of combining chemotherapy agents with CBD. It also provides in vivo evidence that CBD reduces the growth of head and neck tumors as an effective cytotoxic agent against OSCCs. These results show that CBD may target cancer stem cells and reduce chemoresistance so an experiment is conducted to determine the mechanism for its anti-oral cancer effect against cancer stem cells and to increase its chemosensitivity to give more effective treatment.

Recent studies show that it exhibits an anticancer effect [41] and has low toxicity[42] so it is suitable for cancer therapy.

In order to examine cell activity in a cell culture, electric cell-substrate impedance sensing (ECIS) is applied. For adherent cells that are grown on gold arrays, ECIS examines the complex impedance spectrum. ECIS measures the morphological alterations adherent cells undergo in response to various stimuli under particular physiological and pathological circumstances. [43, 44]. By monitoring changes in impedance over time, ECIS is most frequently used to measure the attachment and division of cells in addition to barrier function of a cell monolayer. [45, 46].

This study examines how the morphology and motility of OEC-M1 cells, a line of OSCCs, alter in response to various CBD ECIS concentrations. The cellular responses of OEC-M1 cells are assessed using Annexin V/7-AAD staining tests and AlamarBlue cell viability assays, two common cytotoxicity techniques. Calculating morphological parameters, such as the junctional resistance between adjacent cells ( $R_b$ ) and the average distance between the basolateral cell surface and the substrate, requires comparing experimental data for OEC-M1 cells that cover an electrode with the calculated values for an appropriate cell-electrode model ( $h$ ) [47, 48]. This method provides a more accurate evaluation of morphological changes caused on by apoptosis.

## 2. Materials and Methods

### 3.1. Cell culture and reagents

OEC-M1 cells were cultured in RPMI 1640 media with 10% fetal bovine serum and 1% penicillin/streptomycin at 37°C/5% CO<sub>2</sub> and kept there at the same temperature. When a confluence level reached around 80–90%, cells were routinely sub-cultured. Different CBD concentrations were introduced starting at the 24th hour after planting and continued for 48 hours. 100% dimethyl sulfoxide was used to dissolve the CBD (DMSO). All substances and tools were bought from Sigma-Aldrich (St. Louis, MO, USA).

### 3.2. Impedance Measurement using ECIS

Components of the ECIS Z system were purchased from Applied BioPhysics (Troy, NY, USA). This study made use of 8W1E arrays (8 wells with a 250  $\mu$ m diameter gold sensing electrode). For ECIS, electrodes that are connected by a relay bank to a lock-in amplifier, which monitors in- (real) and out-of-phase (imaginary) voltages across the cell-covered electrode, were used to measure the interaction between the cell and the substrate.

$1.25 \times 10^5$  cells/cm<sup>2</sup> of cells were put into each well. After the cells had attained confluency 24 hours after being seeded, they were given one of the four distinct anti-cancer medications for an additional 24 hours. The impedance of each cell-covered electrode was measured at 25 different frequencies ranging from 31.25 Hz to 100 kHz in order to track changes in cell shape. A cell-free electrode and the same electrode coated with OEC-M1 cells had their impedances tested. Prior to and following cell exposure to various anti-cancer drug doses, the same frequency-scan measurements were made. The junctional resistance between adjacent cells ( $R_b$ ) and the average distance between the basolateral cell surface and the substratum are two morphological parameters that can be determined by comparing experimental data for a cell-covered electrode with calculated values produced by a suitable cell-electrode model ( $h$ ).

### 3.3. Cell viability assay

Cell viability was assessed using the AlamarBlue assay (Thermo Fisher Scientific, Carlsbad, CA, USA) according to the manufacturer's instructions. In brief, OEC-M1 cells were plated to 24-well-plates at a density of  $1 \times 10^4$  cells/well, and let the cell attached for 4 hours. The cultured medium was replaced by medium with CBD and cisplatin at indicated concentrations. Then, the AlarmaBlue reagent was directly added to each well of

the 96-well plates, and cells were then incubated for another 2 hours at 37°C. The fluorescence signals of each well were measured by Synergy H4 Hybrid microplate reader (Bio-Tek Instruments).

### 3.4. Cell apoptosis assay

Cell apoptosis was determined using the Millipore Annexin V/7-AAD staining technique (Billerica, Massachusetts, USA). The majority of the annexin V positive and annexin V/7-AAD double positive cells were found to include apoptotic cells. Cells were plated in 24-well plates at a density of  $5 \times 10^4$  cells per well to test the various treatments' capacity to cause apoptosis in OEC-M1 cells. Cells were given starting doses of CBD and Cisplatin at 10, 30, and 100  $\mu\text{M}$  for 24 h of incubation. Following a PBS wash, trypsinization, and 10-minute centrifugation at 1,200 rpm, the cells were used. After centrifugation,  $1 \times 10^5$  cells were stained using the manufacturer's recommended Annexin V/7-AAD reagents, and flow cytometry was utilized to analyze the results using the Muse® Annexin V and Dead Cell Assay technique (Millipore, Billerica, MA, USA).

### 3.5. Statistical analysis

Statistical analysis uses a Student's t-test and one-way ANOVA. All data is expressed as mean  $\pm$  standard deviation. The level of significance is  $P < 0.05$ .

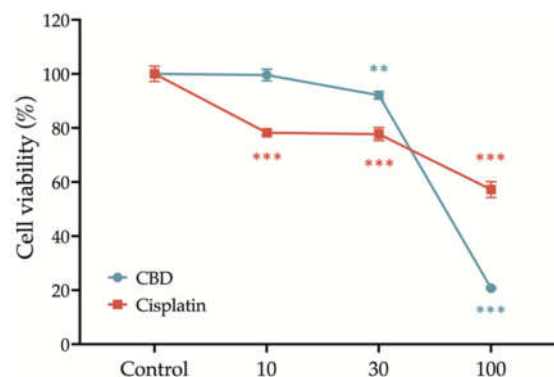
## 3. Results

### 3.1. Effects of CBD and cisplatin on cell viability of OEC-M1 cells

To investigate the anti-cancer properties of CBD on oral cancer, we first assess its cytotoxicity on gingival squamous cell carcinoma cells, OECM-1. Cisplatin, a conventional chemotherapy drug, was used as a positive control in the present study to further confirm the clinical application potential of CBD.

To determine the anti-cancer properties of CBD on oral cancer, cytotoxicity to gingival squamous cell carcinoma cells, OECM-1 was determined. Cisplatin, which is a conventional chemotherapy drug, was used as a positive control for this study to confirm the potential of CBD for clinical application.

After treatment with a series concentration of Cisplatin and CBD for 24 hours, the OEC-M1 cells' viability was determined using an AlarmaBlue assay. Figure 1 shows that cell viability is slightly decreased after treatment with 30  $\mu\text{M}$  CBD and is significantly reduced if the concentration of CBD is increased to 100  $\mu\text{M}$ . Treatment with Cisplatin significantly decreases cell viability at an initial concentration of 10  $\mu\text{M}$  but the cell viability remains at 60% if the concentration is increased to 100  $\mu\text{M}$ . These results show that CBD inhibits the viability of OEC-M1 cells in a concentration-dependent manner and that CBD is more cytotoxic than Cisplatin.



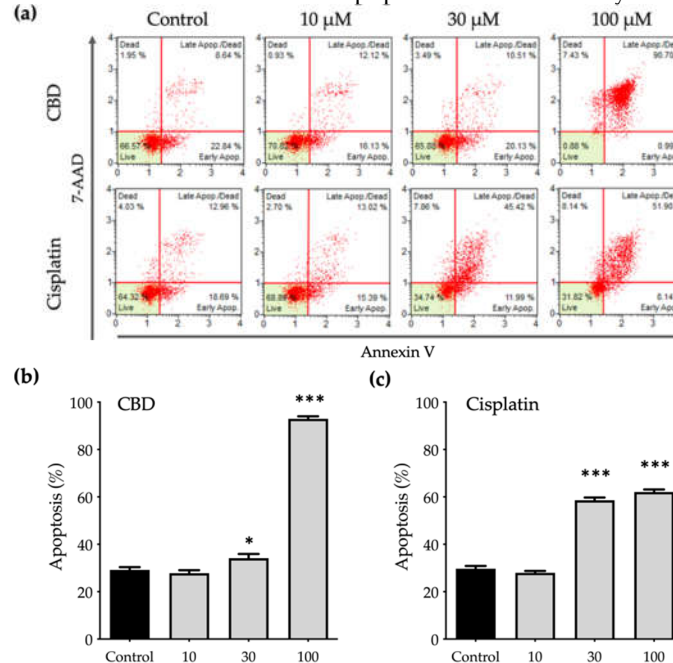
**Figure 1.** Cytotoxic effect of CBD and Cisplatin on the viability of OEC-M1 cells. After the treatment with 10, 30, and 100  $\mu\text{M}$  CBD (blue line) and cisplatin (red line) for 24 hours, the viability of OEC-M1 cells was determined using an AlarmaBlue assay. Untreated cells with an equivalent percentage of DMSO were used as the control group. All data is represented as mean  $\pm$  standard error mean (SEM) for at least three independent experiments. \*\*  $p < 0.01$ ; \*\*\*  $p < 0.001$  compared to control group.

### 3.2. Effects of CBD and cisplatin on cell apoptosis of OEC-M1 cells

Phosphatidylserine (PS) translocation from the inner to the outer leaflet on a cell membrane is a crucial step in apoptosis so the outer PS is detected using Annexin V with fluorescent dye. Eventually, apoptotic cells lose the integrity of the plasma and nuclear membranes and 7-AAD enters the nucleus and binds to the nucleic acids. To determine whether CBD inhibits the viability of OEC-M1 cells by inducing apoptosis, an Annexin V/7-AAD staining assay was used and both Annexin V positive (Annexin V<sup>positive</sup>) and Annexin V/7-AAD double positive (Annexin V<sup>positive</sup>/7-AAD<sup>positive</sup>) populations were considered to be apoptotic cells.

Figure 2 shows that CBD and Cisplatin induce apoptosis in a dose-dependent manner. Treatment with 10  $\mu\text{M}$  Cisplatin significantly decreases the cell viability (Figure 1) without affecting apoptosis (Figure 2c). This decrease in cell viability may be caused by an impairment in function of the mitochondria due to cisplatin, which decreases the power of cells to reduce the AlarmaBlue reagent.

Apoptosis after treatment with 100  $\mu\text{M}$  cisplatin produces a greater Annexin V<sup>positive</sup>/7-AAD<sup>positive</sup> population than treatment with 30  $\mu\text{M}$  cisplatin (Figure 2a), which is positively correlated with lower cell viability. Similarly, to the effect on cell viability, treatment with 30  $\mu\text{M}$  CBD has a significant but slight effect on cell apoptosis, as shown in Figure 2b. When the concentration of CBD is increased to 100  $\mu\text{M}$ , cells almost undergo apoptosis and almost 90% of the Annexin V<sup>positive</sup>/7-AAD<sup>positive</sup> population. These results show that CBD induces cell apoptosis more efficiently than Cisplatin.



**Figure 2.** Apoptotic effect of CBD and Cisplatin on OEC-M1 cells: (a) Apoptosis of OEC-M1 cells following treatment with CBD and cisplatin at 10, 30 and 100  $\mu\text{M}$  was determined using Annexin V/7-AAD staining assays. The cell populations with Annexin V/PI double negative and Annexin V positive and Annexin V/PI double positive are indicated as live and apoptotic cells, respectively. The statistic histogram shows the changes in the apoptotic populations after treatment with (b) CBD and (c) Cisplatin for 24 hours. All data is represented as mean  $\pm$  standard error mean (SEM) for at least three independent experiments of (A). \*  $p < 0.05$ ; \*\*\*  $p < 0.001$  as compared to the control group.

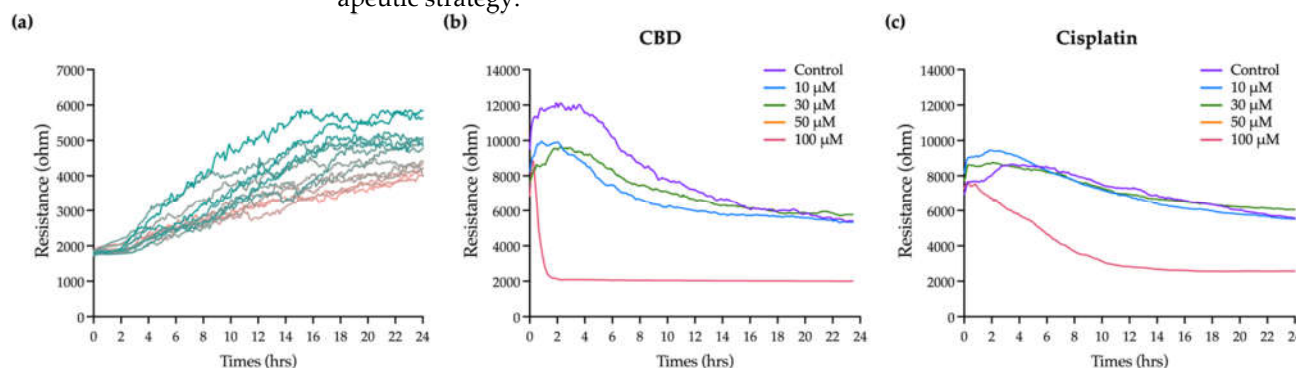


### 3.3. Effects of CBD and cisplatin on the overall resistance time course for OEC-M1 cells

CBD has a significant effect on the viability and apoptosis of OEC-M1 cells, as confirmed the general biochemical assays, but these do not provide comprehensive information on the time-dependent effect because there is only a single end-point measurement. The insulating nature of cell membranes allows information on morphological changes and cell-cell interactions to be obtained by measuring changes in cell impedance. ECIS, which is an impedance-based cellular assay, was used to measure the effect of CBD and Cisplatin on cell morphology and the behavior of OEC-M1 cells in real-time.

Figure 3a shows that the resistance in each well before adding the cells is 2 k $\Omega$ . As cells are seeded, attached, grow and expand on the electrodes, they gradually block the current flow so resistance is increased. Eventually, the resistance curve reaches a plateau at 10 to 20 hours when OEC-M1 cells reach confluence. Minor fluctuations along the resistance curve are caused by cellular micromotion. To obtain the optimal results of cell resistance, current was sequentially passed through the cells at eleven frequencies. The optimal frequency of current that causes the most significant difference in resistance compared to the cell-free group is 4 kHz (data not shown). For this study, all resistance time course data was measured at 4 kHz.

Various concentrations of CBD and cisplatin were added to challenge the OEC-M1 cell layer and changes in the overall resistance of cell layer were measured for 24 hours. Figures 3b and 3c show that, treatment with 10 and 30  $\mu$ M CBD and Cisplatin has no significant effect on the resistance, compared to the control. The cell resistance decreases significantly when it is exposed to 100  $\mu$ M CBD and the lowest resistance (near the value of the cell-free condition, as shown in figure 3a) is achieved within 2 hours. The resistance decreases gradually for 100  $\mu$ M Cisplatin. These results show that ECIS gives more time kinetics information on the cytotoxicity effect than a traditional end-point assay, which is necessary to determine the starting point for the cytotoxic effect and for optimizing a therapeutic strategy.



**Figure 3.** Effect of CBD and Cisplatin on the integrity of the OEC-M1 cellular layer using the ECIS method: (a) The increased resistance time course curve shows the process for the formation of a OEC-M1 cell monolayer. Each resistance curve is for each independent electrode containing wells. (b) CBD and (c) Cisplatin were added into each ECIS well (at time 0) for 24 hours of incubation. The change in resistance compared to the control group shows the integrity of the cell layer after the treatment with the drug. Each curve shows similar results for at least three independent experiments.

### 3.4. Effects of CBD and cisplatin on the morphological parameters for OEC-M1 cells

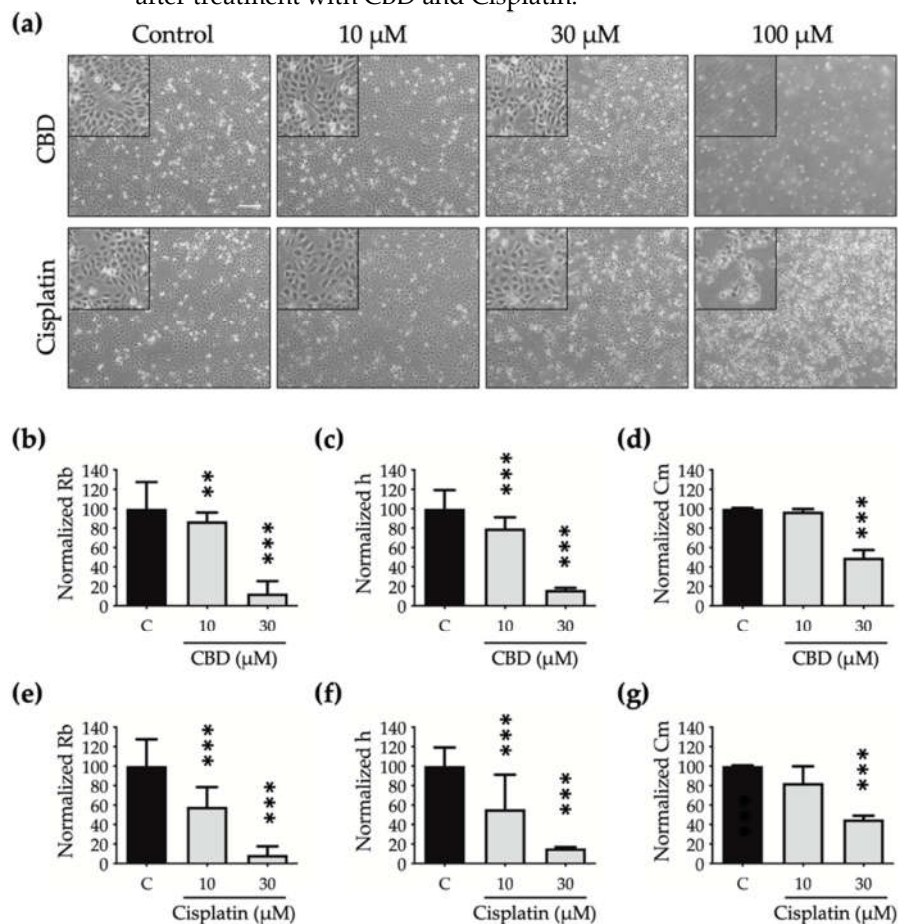
A cell that dies undergoes multiple morphological changes. In Figure 4A, the phase-contrast microscopic images show that the OEC-M1 cells shrink (at 30  $\mu$ M) and then detach as the concentration increases (at 100  $\mu$ M) for both drugs. These are the general characteristics of cells undergoing death. However, the subtle changes in cell morphology are not clearly shown in these optical images.

To determine the effect of CBD and Cisplatin on morphological changes in OEC-M1 cells, particularly in terms of cell-cell interaction and cell-substrate contact, the resistance

time course data is normalized using the cell-electrode model calculation to quantify the change in the cells' morphological parameters, including the value for the junctional resistance between cells ( $R_b$ ), the average cell-substrate separation ( $h$ ) and the membrane capacitance ( $C_m$ ).

OEC-M1 cells almost detach after exposure to 100  $\mu\text{M}$  CBD and Cisplatin for 24 hours so this morphological modelling analysis is inappropriate at this concentration. Figures 4b-g show that the  $R_b$ ,  $h$ , and  $C_m$  values decrease in a dose-dependent manner after treatment with CBD and Cisplatin. The values for  $R_b$  (Figures 4b and 4e) and  $h$  (Figure 4c and 4f) are less than those for cells that are treated using Cisplatin so the edge and is blurred and the shape is flattened.

There is also a significant decrease in the  $C_m$  value for cells that are treated with CBD and Cisplatin (Figures 4d and 4g) which shows that the cell membrane shrinks after treatment with both drugs and the cell volume decreases. Quantitative data for multiple cell morphological parameters analyzed by ECIS shows the different toxic effects on cell shape after treatment with CBD and Cisplatin.

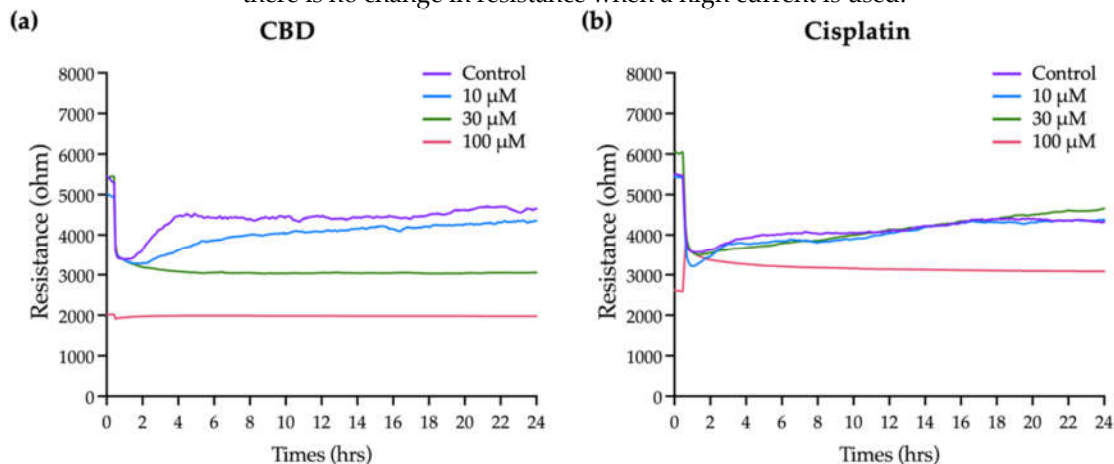


**Figure 4.** Effect of CBD and Cisplatin on the cell morphology of OEC-M1 cells: (a) Images of OEC-M1 cells that are exposed to CBD and cisplatin at 10, 30, and 100  $\mu\text{M}$  at the 24th hour from a phase-contrast microscope. Scale bar; 200  $\mu\text{m}$ . The changes in the morphological parameters of the normalized junctional resistance between cells ( $R_b$ ), the average cell-substrate separation ( $h$ ) and the membrane capacitance ( $C_m$ ) were measured using the cell-electrode model calculation for cell resistance data. The histogram shows the changes in the normalized  $R_b$ ,  $h$  and  $C_m$  values for OEC-M1 cells after treatment with (b-d) CBD and (e-g) Cisplatin at 10 and 30  $\mu\text{M}$  for 24 hours. All data is represented as mean  $\pm$  standard error mean (SEM) for at least three independent experiments. \*\*  $p < 0.01$ ; \*\*\*  $p < 0.001$  as compared to control group.

### 3.5. Effects of CBD and cisplatin on the cell migration and micromotion of OEC-M1 cells

Cancer cells migrate easily so invasion and metastasis are common. An ECIS wound-healing assay was conducted to determine the effect of CBD and Cisplatin on cell migration. A high current was used to annihilate cells on the electrodes, so resistance decreases significantly, as shown in Figure 5 (black arrows). The subsequent recovery of cell impedance shows that surrounding cells migrate and cover the electrodes.

As shown in Figure 5b, when a high current is used, the resistance recovery gradients for cells that are treated with 10 and 30  $\mu\text{M}$  cisplatin are similar to those for the control group. The resistance does not recover at 100  $\mu\text{M}$  cisplatin, so 100  $\mu\text{M}$  cisplatin completely inhibits the migration of OEC-M1 cells. As shown in Figure 5a, treatment with 10  $\mu\text{M}$  CBD has no significant effect on resistance recovery. Treatment with 30  $\mu\text{M}$  CBD has a limited effect on cell viability (Figure 1) but completely inhibits the resistance recovery gradient. These results show that cell migration due to treatment with 30  $\mu\text{M}$  CBD is inhibited before the induction of apoptosis. Cells that are treated with 100  $\mu\text{M}$  CBD are almost dead so there is no change in resistance when a high current is used.



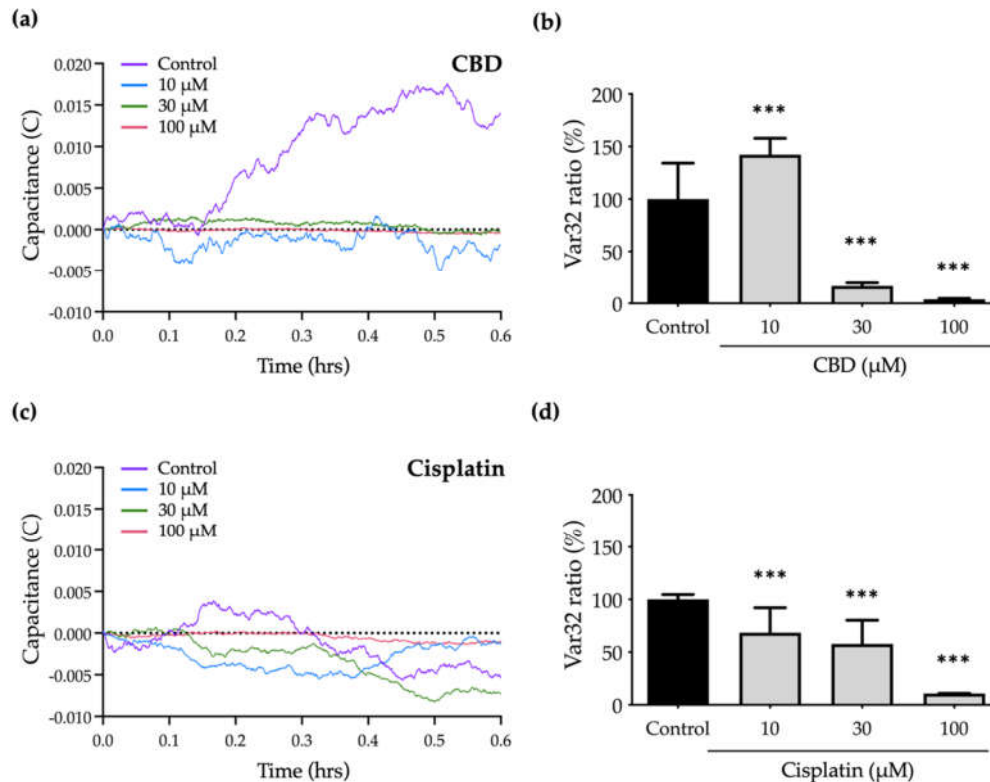
**Figure 5.** Effect of CBD and Cisplatin on the migration of OEC-M1 cells. The wound area of the cell layer on the electrode is created by passing a relatively high current (at time 0, indicated by the black arrow). Subsequent resistance recovery gradients show the migration of OEC-M1 cells after exposure to (a) CBD and (b) Cisplatin at 10, 30, and 100  $\mu\text{M}$ . Each curve contains similar results for at least three independent experiments.

To determine the effect of CBD and Cisplatin on the micromotion of OEC-M1 cells, a numerical analysis is used to characterize the normalized capacitance (C) time courses and to calculate the Var32 values. The fluctuations in the C curve for treated cells are smaller than those for the control group because cell micromotion is inhibited. Figures 6a and 6c show the significant decrease in C curves in response to treatment with CBD and Cisplatin.

To quantify the cell micromotion, the normalized capacitance (C) time course values are analyzed using the Var32 calculation model. The average cell micromotion is represented as the Var32 ratio. Treatment with 10 and 30  $\mu\text{M}$  cisplatin has no effect on cell migration, compared to the control group (Figure 5b), but the Var32 ratio is significantly decreased, so micromotion is inhibited (Figures 6c and 6d). Treatment with 30  $\mu\text{M}$  CBD results in a significant decrease in the Var30 ratio (Figures 6a and 6b), which is consistent with the complete inhibition of cell migration by CBD at the same concentration (Figure 5a). However, the Var32 ratio increases if the cells are exposed to 10  $\mu\text{M}$  CBD.

These results show that treatment with 30  $\mu\text{M}$  CBD significantly inhibits cell motility but has a lesser effect on cell viability, so it is a specific anti-metastasis compound. The data for this study also shows the advantages of ECIS for the analysis of cell migration and micromotion in that the effect of anti-cancer drugs on metastasis-related abilities at different levels of cellular behaviors can be determined.





**Figure 6.** Effect of CBD and Cisplatin on micromotion of OEC-M1 cells: (a) The fluctuation in the capacitance (C) values time course shows the micromotion of OEC-M1 cells after treatment with (a) CBD and (c) Cisplatin for 24 hours. The C values are normalized and analyzed using the Var32 calculation model, and the average cell micromotion is the Var32 ratio. The histogram shows the changes in the Var32 ratio for OEC-M1 cells that are treated with (b) CBD and (d) Cisplatin at 10, 30, and 100  $\mu$ M. All data is represented as mean  $\pm$  standard error mean (SEM) for at least three independent experiments. \*\*\*  $p < 0.001$  as compared to control group.

#### 4. Discussion

Local or regional invasion is frequently seen in oral cancer patients. Because of the abundance of vascular and lymphatic veins in this region, oral cancer has a higher potential to spread to other parts of the body. Chemotherapy and surgical resection are used to treat oral cell carcinoma that has migrated or recovered, although the mortality rate is significant.

Studies reveal that individuals receiving the usual chemotherapy medicines 5-FU [49] and Cisplatin together exhibit a greater overall response and the malignancy develops more slowly. The effectiveness of any treatment is constrained because while some patients show a particular improvement, other investigations reveal that they also exhibit clinical toxicity. There is a need for an alternate treatment approach since oral malignancies are aggressive and highly proliferative. Like traditional chemotherapy medications, pharmaceuticals must follow an inherent apoptotic route.

This investigation explores the effects of the natural substance CBD. Previous research have demonstrated that CBD therapy inhibits the invasion and migration of various cancer cells. [41, 50]. The concentration at which CBD has an inhibitory effect is determined by measuring cytotoxicity and cell viability using AlamarBlue [41]. ECIS is a real-time label-free monitoring technique used to assess the best efficiency at various concentrations [48, 51, 52]. In general, OEC-M1 cells exhibit an initial stabilization phase, followed by a second stabilization phase following drug inoculation.

When confluency is reached, the resistance stabilizes at 5–6 k $\Omega$  from the first phase's low resistance of 2–3 k $\Omega$ . Because the OEC-M1 cells enlarge a little following CBD

treatment, they are all more resistant to the medication than the control group. The cytoplasm shrinks and the cell shape changes when OEC-M1 cells are exposed to sufficiently high concentrations of CBD (30  $\mu$ M) or Cisplatin (10  $\mu$ M), resulting in varying degrees of reductions in the overall resistance time courses.

Biochemical experiments were performed in order to determine the morphological alterations associated to the induction of apoptosis by anticancer substances or medications utilizing ECIS. Previous research has demonstrated that CBD exposure inhibits the invasion and migration of various cancer cells. AlamarBlue's analysis of cell viability reveals the considerable inhibitory doses for both medications. There is a link between these two measurements since the profile for cell viability (%) at various concentrations is comparable to that for the Var32 ratio.

This study uses the Annexin V staining and flow cytometry to examine if the anti-proliferative action of cells following drug treatment is dependent on apoptosis. According to the results, both drugs cause an increase in the quantity of apoptotic cells that is concentration-dependent when compared to control groups. These biochemical data match with the ECIS findings. According to the results of a study, CBD has a more lethal effects on OEC-M1 cells' viability at 100  $\mu$ M and migration at 30  $\mu$ M when compared to cisplatin at the same concentration. This is because apoptosis causes changes in cell morphology, micromotion, and cell viability. This work also shows how to evaluate chemotherapy drugs using an impedance-based cellular assessment. Since ECIS is sensitive to pharmacological and physiological changes, it is a suitable first-passage testing ground for novel medications.

#### 5. Conclusions

This study determines the effect of CBD on OEC-M1 cells. The cytotoxicity results show that CBD at higher concentrations (100  $\mu$ M) increases cytotoxicity and is more likely to lead to the apoptosis of cancer cells more than Cisplatin at the same concentration. ECIS is used to determine the effect of the drug on the adhesion, spread and migration of cells. The results show that there is a linear, concentration-dependent decrease in OEC-M1 cells that are treated with CBD. Treatment with CBD at low concentrations (30  $\mu$ M) completely inhibits cell migration and micromotion without affecting cell viability and apoptosis. This study shows that CBD may be a therapeutic drug for oral cancer.

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