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Characterization by Electric Impedance Sensing of Normal and Cancer Cell Attachment and the Effect of Ginseng

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Abstract: This research reestablishes an application of an Electric Cell Substrate Impedance Sensing (ECIS) system to examine the effects of Ginseng on the attachment of epithelial cells; HaCAT (healthy human skin cells) and A431 (human carcinoma cells). The study is functionally a continuation of the authors' previously published paper, where an identical set of experiments were carried out for the putative homeopathic Reishi. The purpose for this study was two-fold: (1) to investigate variations in results between a roundly discredited homeopathic (Reishi) and a studied, well-accepted homeopathic (Gensing), and (2) to justify the experimental technique from the results - as the electric impedance sensing technique has heretofore been considered itself experimental; specifically, impedance measurements reflecting cell-to-cell binding and their associated binding electric field strength and the high correlation to shape (normal vs. cancer) and cluster size (as measured by increased field strength in greater clusters). Here the impedance analysis shows that Ginseng at applied doses does not alter the impedance of HaCAT colonies but that Ginseng does significantly increase the strength of cell-to-cell attachments in A431 colonies and may confer previously suggested anti-inflammatory properties. This result is not seen with the previous set of Reishi experiments, and this correct distinction provides more ammunition for the electrical impedance sensing technique as a valid differentiation in cell attachment.

Keywords: Cell attachment, Cell membrane, Epithelial cells, Ginseng, Electric impedance sensing

Introduction

The attachment of epithelial cells is essential in maintaining structure and scaffolding for biological tissue. More than seventy percent of cancers are related to epithelial cells and layers [1-2]. The function of the epithelia may play a role in cancer progression [3]. Normally, epithelial sheet layers replace apoptotic cells without altering the barriers activities [4-9]. However, cancer cells may affect this regeneration process and leak through the epithelial sheet [10]. These cancer cells can cross the protective tissue and penetrate into the body fluid stream, which results in metastasis.

Ginseng is an herbal medicine commonly used to treat variety of illnesses in Asia such as cancer, diabetes, immune system disorders, neuronal, cardiovascular, liver, and infectious diseases [11-21]. Several studies have revealed its anti-cancer, anti-inflammatory, and immune-modulatory effects [11, 13, 22]. Ginseng is comprised of tetracyclic triterpenoids (ginsenosides), polyacetylenes, polyphenolic compounds, and acidic polysaccharides. The health benefits of ginseng are attributable to its main pharmacological component ginsenoside [13]. Ginsenoside Rp1 has reportedly shown anti-cancer effects and suppressed the cell growth of 21S and HeLa cells [11, 23]. Additionally, melanoma cells transplanted in a mouse treated with ginseng G-Rb2 have been reported to inhibit tumor growth and angiogenesis [11, 24]. A further study has reported on ginseng improvement of mesenchymal cell attachment [12].

This pilot study examines the effects of Ginseng on the attachment of healthy human epithelial cells (HaCAT) and cancer cells (A431) using an electric impedance sensing system and contrasts results with a previously studied homeopathic (Reishi) to validate the experimental technique. Further, this study develops the intrinsic relationship between cell morphology and impedance.

Method

Cell Preparation

For the experiments, two epithelial cell lines: (1) HaCAT cells derived from normal human skin and (2) A431 cells derived from human carcinoma cells (ATCC) were used. Both HaCAT and A431 cells were treated with a medium solution made with Dulbecco's Modified Eagle's Medium (DMEM, obtained from ATCC), 10% Fetal Bovine Serum (FBS, obtained from WVR), one ml 2.5 μg/ml Fungizone (obtained from Fisher), and 50 μg/ml Gentamicin (obtained from Fisher).

For a detailed description of the cell preparation methodology, please refer to the 'Experimental Method' Section, 'Cell Preparation' Subsection of the authors' previous publication [25]- as the cell preparation is purposefully identical such that results may be compared directly for two distinct homeopathics (Reishi, Gensing).

Further, precise replication of cell preparation allows for verification of the consistency in results for the electric impedance system - heretofore unverified as a tool specifically directed to measuring cell-to-cell binding strength.

Ginseng Preparation

A Red Panax Ginseng root vial 10 cc, 500 mg (Red Ginseng Royal Jelly) was used for examining its effect on cell attachment. Each vial consisted of red panax ginseng root (500 mg) and royal jelly (350 mg). Other ingredients include purified water, honey, alcohol (less than 0.5%), and potassium sorbate (preservative). The 10 cc ginseng solution was added to 40 cc DMEM medium and mixed for one hour at slow speed and low heat using a magnetic stirrer. The stock solution was filtered using a sterilized filter (Fisher Scientific). Then, the 10 mg/mL ginseng stock was refrigerated. For the experiments, the ginseng at doses of 0.005, 0.01, and 0.02 mg/mL was used.

Electric Impedance Sensing System

For impedance measurements, an electric impedance system called electric cell-substrate impedance sensing (ECIS) was obtained from Applied BioPhysics. Cell spreading and cellular attachment is known to change the impedance (limit current flow) between a basal membrane and the supporting substrate [26, 27]. As with cell preparation - the details for the ECIS System are provided in the authors' previous work [25] and are identically replicated in the current work such that all possible variants are eliminated with the exception of the tested homeopathics (Reishi, Gensing).

Results and Discussion

The resistance and capacitance of HaCAT cells and A431 cells were analyzed at 4 and 64 kHz, respectively. Note that these frequencies exactly match the frequencies associated with the authors' previous work [25].

Figure 1 and 2 show the resistance and the capacitance of both cell types. In the first 3-hour period, cell spreading allow current through the cellular network while DMEM concentration is increasing and settling closer to the electrodes. At hour 3, the resistance and capacitance reach a regional minimum and maximum.

After 3-hours, the resistance increases abruptly and the capacitance decreases rapidly for the next 16 hours, then both reach new levels, and spike quickly after DMEM refresh and ginseng treatment (concentrations: 0.005, 0.01, and 0.02 mg/mL) at hour 19 - at which point the cells were examined out of the incubator by microscope to ensure a confluent layer. Resistance and capacitance levels then continue in decent and ascent, respectively, for HaCAT cells whereas after the spike at hour 19 both impedance levels remain flat for A431 cells - at least until hour 29.

The DMEM refresh and ginseng treatment showed distinct effects on the resistance and capacitance - for both normal cells and cancer cells. For HaCAT cells, the resistance and capacitance plots converge at least through hour 29, following a different pattern and slope from what was found in the 19 hours just prior to refresh. The impedance measurements return to equilibrium levels and remain nearly flat after hour 55. For A431 cells, the

resistance and capacitance show stronger spikes than detected in HaCAT colonies, but both measurements quickly return to steady-state levels through hour 29 and – unlike the impedance measurements for HaCAT colonies - impedance levels for disparate ginseng concentrations do not converge.

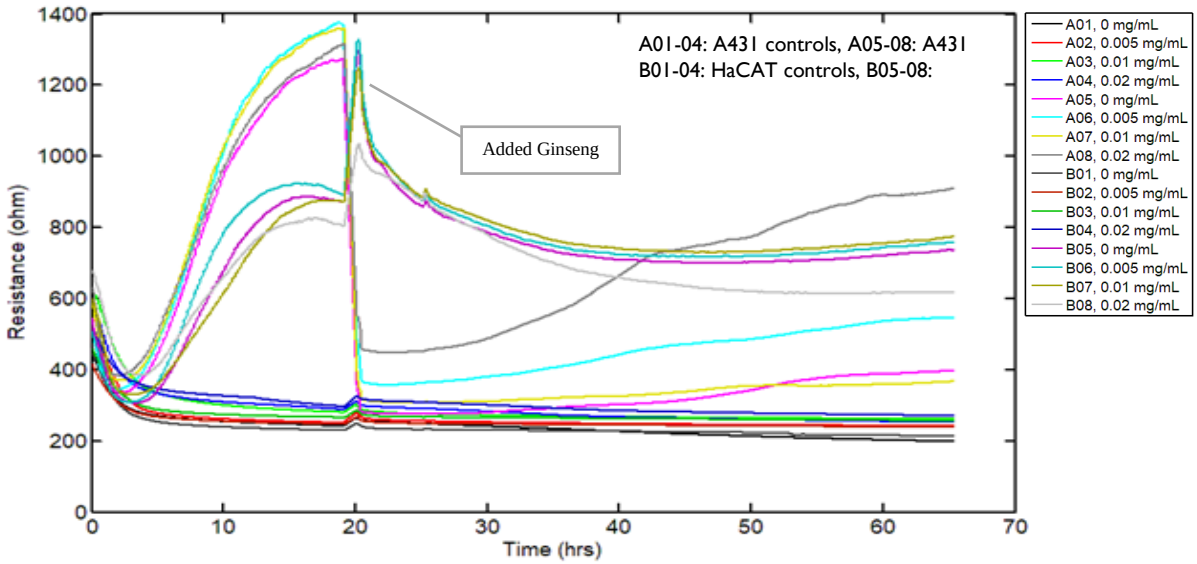

Figure 1. The Resistance of A431 Cells (4 increasing lines at the top before hour 20), HaCAT Cells (4 increasing lines in the middle before hour 20), and Controls (8 lines at the bottom).

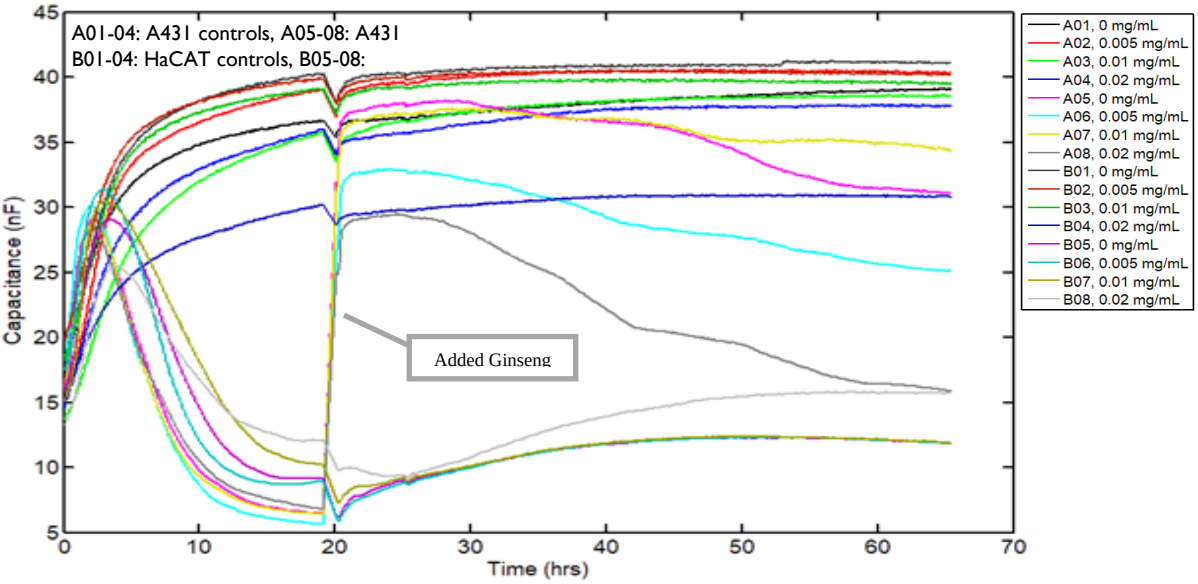

Figure 2. The Capacitance of A431 Cells (4 declining lines in the middle after hour 20), HaCAT Cells (4 lines on the bottom after hour 20), and Controls (8 lines at the top).

A brief but detailed description of the how and why electrical impedance measurements are correlated to cell morphology and structure can be found in the authors' previous work [25].

A brief summary of the experimental results for Ginseng additives follows. Results found in a 3-hour start-up cycle and ensuing 19-hour period to reach cell attachment equilibrium in the Ginseng experiments exactly mirror the start-up and equilibrium results found in the Reishi experiments. Following DMEM refresh, electrical impedances spike, reacting to the immediate impact of an added nutrient and the jostling of cell attachments from the physical act of shutting down and starting up the experiment. Specifically, resistance measurements for HaCAT cells spike up where A431 resistance spikes down - control wells (no cells) resistance remains constant. Reports that Ginseng effectively enhances cellular attachment [12] may tell only part of the story; this effect appears to be short-lived and dependent on cell morphology.

After spiking in response to DMEM refresh and Ginseng treatment at the 19-hour point, HaCAT cells resistance and capacitance decrease and increase a few percent, respectively, and remain nearly constant after hour 40. By contrast, A431 impedance curves sharply change to levels just after start-up, and stay flat - at least until hour 29. Afterward, the resistance and the capacitance start to incline and decline, respectively, at a slower rate.

A431 impedance drops (hour 20) are related to the metabolism and morphology of cancerous cells. Following a rapid decrease in the electric field strength at the cell-cell interface -and a correlated charging of the cell body- the impedance levels quickly return to equilibrium levels. After returning to the equilibrium level, the cell matrix is efficiently reassembled -the entropy is decreased. New bonds are made slowly to the new arrangement.

At hour 29, the resistance levels of A431 demonstrate their stable ascent at much lower rate compared to those found just after the first 3-hour period. This is correlated to a weakened A431 cell-to-cell attachments. However, the well treated with 0.02 mg/mL ginseng shows a much faster increase in resistance and decrease in capacitance than lower concentrations. This is significant.

The rapid change in impedance could be attributed to the anticancer effect of ginsenoside [11,13] and morphology and cytoskeletal change by steroid [28], which may have resulted in the breaking of cell-to-cell bonds.

The most important takeaway from Figures 1-2 is the stark effect a greater ginseng level (0.02 mg/ml) had on the relative bulk resistance and capacitance of cancer cell colonies. Specifically, the greater concentration of ginseng additive produced greater resistance and reduced capacitance measurements in A431 colonies, but had little to no effect on normal cell colonies and the control.

By comparison, similar experiments conducted by this group [25] demonstrated no change in A431 impedance levels - nor changes in HaCAT nor control impedance levels for that matter - with the addition of similar concentrations of Reishi.

As described previously [25], charges stolen from the cell bulk to service an increasing electric field at the more sharply pointed, highly curved cancer cell boundary strengthens cell-to-cell bonding and is manifested by both a sharp increase in resistance and a sharp drop in capacitance.

For HaCAT colonies, the DMEM refresh results in a charge rearrangement in cellular network and reinforced bonds between cells. The impedance values cross between hours 21-30 and reach to nearly flat levels. This indicates that separate wells now are related by a common factor, which is manifested by a steady level for the impedance.

Conclusion

This study found a distinct impedance response at frequencies 4 kHz and 64 kHz for normal cells and cancer cells, in agreement with the authors' previous publication [25]. And, as before, this study demonstrates that both cell spreading and cell-to-cell attachment affect impedance measurements, and that cell behavior can be determined from impedance data. Importantly, to emphasize a point made in the authors' previous publication [25], both cell spreading and attachment mechanisms observed here are reflected at the tissue-epithelial lining. The impedance variation (rise in the resistance and decline in the capacitance levels) at the 19-hour point is related to an increasing electric field, giving rise in a tighter network of cell-to-cell attachments. Each of these findings exactly match findings in the authors' previous work [25] before the introduction of homeopathic additives.

The nonuniform shape of cancer cells and the strong electric field in cancer cells holds them in place. A significant weakening, repositioning and reattachment is detected in the impedance data after DMEM refresh.

The repositioning and reattachment of healthy epithelial cells due to DMEM refresh and disturbance is also reflected in biology as cells react to both nutritional requirements and repositioning along the epithelial lining.

This set of experiments depicted ginseng at doses of 0.005, 0.01, and 0.02 mg/mL initially increases the impedance but shortly after refresh the impedance of normal cell colonies (HaCAT) returns to an equilibrium level. A431 resistance and capacitance levels change markedly at the highest ginseng concentration, unlike the HaCAT colonies and controls.

This relative impedance change may be related to the steroid (ginsenosides) and sugar (oligosaccharides) content in ginseng. Cancer cell morphology and metabolism may be linked by the Warburg Effect [29], providing for a strengthening of A431 cell-to-cell bonds and a corresponding increase in bulk resistance and decrease in bulk capacitance measurements.

Ginseng is widely viewed as an anti-inflammatory and antioxidant, providing an increased immune response and some level of resistance to cancer onset. But what if the mechanism is counterintuitive. Instead of isolating cancer cells, ginseng may 'lock' cancer cell chains so tight that epithelial cell and tissue penetration is more difficult due to the sheer size of the cancer cell mass. The impedance measurements taken in this study point to a tighter collection of cancer cell colonies with the addition of ginseng, an unexpected and surprising result.
Further impedance experiments with higher dosages of ginseng could reveal more insight on the effects of ginseng-effects that could validate ginseng for therapeutic homeopathic use.

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