Detection of Mouse Mammary Tumor Virus (MMTV) Particles in an Immortalized T Cell Line based on Electrical Parameters

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ABSTRACT Label-free detection and characterization of cells producing virus particles is a highly desirable property that can pave the way for direct detection of virally-infected cells in body fluids, tissues and eventually in infected individuals. Identification of such properties can also provide a better understanding of the growth and/or evolution of virally-infected cells in real time experimental setups. This work takes a closer look at the electrical properties of an immortalized T cell line capable of producing virus particles along with its non-virus producing control cell line using capacitance-voltage measurements. In addition, two other important control cell lines were also included in which the ability of the cells to produce virus particles was abrogated by using genetic mutations. The conducted capacitance voltage measurements revealed that it is possible to electrically differentiate between these different cell lines. The cells producing wild type (WT) virus particles could be distinguished from the control cells in which no DNA was introduced and hence were incapable of producing any virus particles. Interestingly, the cells in which two mutant DNAs were introduced that abrogated their ability to produce virus particles showed a similar electrical profile to each other, yet distinct from that of the cells producing the WT virus. These results clearly demonstrate that the electrical technique was able to distinguish between cells expressing virus particles versus cells that do not express virus particles. Data analysis revealed a two-fold difference in the interaction capacitance between WT and mutant cells. Characterizing cells using electrical parameters is not laborious and lengthy, as is the case with conventional biochemical methods that typically take several days. Together, these data suggest that it is possible to electrically characterize and differentiate virally-infected cells from uninfected cells. It is our hope that eventually these observations could be translated into label-free, electrical detection of virally-infected cells in biological samples and individuals for diagnostic purposes.

INDEX TERMS Capacitance, capacitance-voltage, cell biology, electrical characterization, detection, MMTV, sensors, virus infection, voltage

I. INTRODUCTION

Viral infections can lead to chronic diseases such as hepatitis, cancer and immunodeficiency syndromes, leading to death if not diagnosed and treated in a timely manner [1]. Polymerase chain reaction (PCR)-based technologies remain the gold standard for the detection and identification of many DNA and RNA viruses. Although very sensitive, these approaches are time consuming and expensive, warranting the need for developing alternate user-friendly detection methods [2, 3]. Viruses are naturally resilient biomolecules with distinct morphological structures that can be polarized by an electric...
field. This produces capacitance values, resulting in unique identifiers or signatures specific for a virus [4]. Understanding the intrinsic properties of viruses using electrical parameters could enable the design of label-free, simpler bioassays [5]. Recently, we reported our preliminary work on label-free identification of viruses based on electrical parameters [5, 6]. Briefly, this was accomplished with capacitance-voltage measurements to quantitate and identify a single virus suspension in culture medium without any pre-processing and/or lengthy preparations. The corresponding virion electrical capacitance was used to identify the kind of virus with the integration of a medium-contributing de-embedding step [6].

Both cells and viruses are considered as complex biomolecules composed of proteins, carbohydrates, nucleic acids, and lipids, in addition to other minor constituents [7]. Normally, such biomolecules get polarized when subjected to propagation of an electrical field [5, 6]. The strength of polarization depends on the composition and interaction with the medium and consequently, any change in cell composition should be reflected in their electrical characterization and measurement [8]. The work presented here is a continuation of this work and investigates the use of label-free electrical capacitance-voltage techniques to distinguish virus-producing cells from those not producing virus.

**THE ELECTRICAL CAPACITANCE MODEL**

The model used to investigate the electrical properties of a virus-producing cell is depicted in Fig. 1. Figure 1(a) illustrates the distribution of a cell suspension inside a capacitive structure. Usually the cell distribution inside the suspension medium is random. For the sake of modeling, the cell distribution inside a capacitive structure can be considered as two separate zones, visualized either as a parallel (Fig. 1(b)) or series model (Fig. 1(c)). The corresponding electrical capacitance models are illustrated in Fig. 1(d) and 1(e), respectively. The volumes of the cell and medium zones are the same as their respective actual volumes in the suspension in both the parallel and series representations. If the effective capacitance of the suspension was lower than the control, than a “series model” should be considered, otherwise a “parallel model” should be considered. Furthermore, Fig. 1(f) represents the virus particle distribution inside a cell. If the cell membrane is sandwiched between two electrodes, the cytoplasm can act as the medium and the viral components can be the complex particles inside the medium; therefore, the illustrations of Fig. 1(a) to (e) can be used to model virus-cell interactions in either a parallel or a series mode of operation.

**FIGURE 1:** Schematic representation of the model used to characterize the electric properties of a virus-producing cell. Illustration of (a) a random distribution of cells in medium; (b) a two-zone parallel model; (c) a two-zone series model. Panels (d) and (e) illustrate the corresponding electrical equivalent parallel and series capacitance models, respectively. Panel (f) illustrates the distribution of virus particles inside a cell. L is length of the total capacitor; d is diameter of the capacitor; Lm and Lc are lengths of the medium and the cell zones, respectively. dm and dc are diameters of the medium and the cell zones, respectively. Cs, Cm, and Cc are capacitance of the solution, medium, and cell, respectively.
II. MATERIALS AND METHODS

A. BIO REAGENTS

Human Jurkat T cells were a gift from Prof. Gulzar Khan, Department of Microbiology and Immunology, CMHS, UAEU. They were cultured in RPMI media supplemented with 10% fetal bovine serum (FBS), 100 units/ml penicillin-streptomycin, and 50 μg/ml gentamycin antibiotics (complete media), as per manufacturer’s specifications, and maintained at 37°C in a 5% CO2 incubator. The HYB MTV plasmid used to express a molecular clone of the mouse mammary tumor virus (MMTV) was a gift from Prof. Jaquelin P. Dudley, The University of Texas at Austin, USA [9]. The anti-p27Gag Blue 7 antibody was a gift from Prof. Tatyana Golovkina, The University of Chicago, USA [10].

B. MUTANT VIRUSES

The wild type HYB MTV plasmid was used to create the two deletion mutations in Mutants A and B [11]. The mutations were introduced into HYB MTV by using splice overlap extension polymerase chain reaction (SOE PCR) as described earlier [11, 12]. Briefly, pairs of mutant sense (S) and anti-sense (AS) primers (Table 1) were used to introduce deletions within the 5’ untranslated region (5’ UTR) of the viral genome, in between the major splice donor and beginning of the gag gene, to abrogate virus gene expression.

Table 1: PAIRS OF MUTANT SENSE (S) AND ANTI-SENSE (AS) PRIMERS OF THE VIRAL GENOME

<table>
<thead>
<tr>
<th>Name</th>
<th>Mutant A</th>
<th>Mutant B</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCR A</td>
<td>OTR 861(+)</td>
<td>OTR 861(-)</td>
</tr>
<tr>
<td>PCR B</td>
<td>OTR 879(+)</td>
<td>OTR 880(-)</td>
</tr>
<tr>
<td>Inner Primers</td>
<td>OTR 878(-)</td>
<td>OTR 881(-)</td>
</tr>
<tr>
<td>Inner Primer Sequence (5’ To 3’)</td>
<td>CCA ATG GCT CCT CTT CTC CCG AGG CCG</td>
<td>GAC CCC CAT ACC GTA ACC TAC CTC TTC</td>
</tr>
<tr>
<td>Genome Position (in nt)</td>
<td>1482-1447</td>
<td>1493-1455</td>
</tr>
</tbody>
</table>

C. CONSTRUCTION OF STABLE CELL LINES

Stable cell lines expressing either the WT or mutant viral genomes were created by electroporation. Briefly, 40 μg of the respective plasmid DNA purified via Qiagen Maxi columns (Qiagen, GmBH) was electroporated into 107 human Jurkat T cell/ml using the BioRad electroporator Gene Pulser II at the following conditions: 950 μF/260 V using the 0.4 cm BioRad cuvettes. The cells were placed on ice for recovery immediately after electroporation for 15 minutes followed by their culture at 37 °C in the RPMI complete media in a 5% CO2 incubator. After 2-3 days, the cells were selected for stable virus expression using RPMI complete medium containing 800 μg/ml of hygromycin B (Hyclone) for 2-3 weeks since the plasmid backbone contained the hygromycin B phosphotransferase gene expressed from the herpes virus TK promoter.

D. WESTERN BLOT ANALYSIS

To determine whether the mutations introduced had indeed abrogated the ability of HYB MTV to express viral proteins, Jurkat stable cell lines expressing the appropriate plasmids were washed once with 1 x phosphate buffered saline (1X PBS) and lysed in 1 ml RIPA buffer (10 mM Tris-Cl (pH 8.0), 140 mM NaCl, 1 mM EDTA, 1% Triton X-100, 0.1% sodium deoxycholate, 0.1% SDS,) per million cells supplemented with 50 μl of β-mercaptoethanol/ml RIPA and 1 mM of the serine protease inhibitor, phenylmethylsulfonyl fluoride (PMSF) (Sigma). Cell lysates were separated from the nuclear pellets and either used immediately or stored at -80°C. Protein amount in the cellular extracts were quantified using the BioRad Bradford colorimetric assay (Bio-Rad, USA). Total cellular protein lysates (50 μg per lane) or BioRad Kaleidoscope molecular weight markers (10 μls) were loaded onto 10% SDS-polyacrylamide mini gels (BioRad) and blotted onto Protran nitrocellulose membranes (Whatman, USA) using standard protocol. After wet transfer, the membranes were blocked with 5% low fat dried milk in 1 x PBS and0.1% Tween-20 (PBST) for 1 hour. The membranes were then incubated at 4oC overnight with the primary antibodies (1:100 dilution of anti-p27Gag Blue 7 [14] or 1:25.000 dilution of anti-actin, Sigma, USA) in 1% milk in PBST. The blots were then incubated with the appropriate secondary antibodies (1:10,000 dilution of anti-mouse-HRP or anti-rabbit-HRP; Sigma, USA) for 1 hour and 30 minutes at room temperature followed by detection using the Pierce™ ECL Plus Western Blotting Substrate and visualized using the Typhoon reader.

III. RESULTS

A. EXPERIMENTAL DESIGN AND ELECTRICAL MEASUREMENTS:

To determine whether the electrical technique being proposed and employed could differentiate between a cell that was producing virus versus a cell that was not producing virus, a cell line was engineered that that could make wild type (WT) virus particles from a plasmid DNA stably integrated into the host cell genome (see Materials and Methods for details). Employing the same methodology, two other cell lines were constructed using viral DNAs containing two different independent mutations that disabled the ability of the viral genome to make virus particles (Mutants A and B, respectively). Cells from the three cell lines were suspend in cell growth media to create cell suspensions. The cell suspensions were then tested by the electrical technique along with either 1) media alone, or 2) cells that did not get any DNA (referred to as “control” from here onwards). The electrical measurements were conducted using Gamry Reference 3000 instrument (Gamry, USA). The capacitance measurements were conducted in a coaxial adaptor connected to coaxial cables. The structure of the coaxial adaptor comprises of inner and outer conductors with dimensions of 2 and 5 mm, respectively, and a length of 7...
The media or various cell suspensions (prepared in media) were loaded into the coaxial adaptor where the media acted as the dielectric material. The advantage of the coaxial topology is that the radiofrequency signal and the electrostatic field propagations are confined and protected from outside interferences and the signals does not escape the space between the inner and outer conductors [5, 6].

B. ELECTRICAL RESULTS
To characterize the three types of cells electrically using the capacitance-voltage-based extracted parameters, we first determined whether the cells in media or media alone were electrically polarizable or not. The charging profiles for the media alone, control cells, and wild type virus-producing cells (referred to as “WT”) are shown in Fig. 2(a). The charging was conducted at a constant current flow for over 300 seconds that demonstrated a stable saturated behavior. The charging voltage was recorded with a step of 1 second. As expected, the medium alone consistently exhibited lower voltage values over the course of charging compared to the cell suspensions since the voltage level depends on the presence and concentration of cells. With the application of a DC current, the suspended cells were polarized and held the charges, thus increasing the voltage compared to the media alone where the voltage stayed constant. The voltage reached its maximum value for the virus expressing cell suspension (WT) when compared to the normal cell suspension (Control). We interpret this data to suggest that the presence of virus within the cell enhanced its ability to hold more charge (Fig. 2(a)) since equal numbers of cells were tested in this experiment.

Next, impedance was measured for the same samples and is shown in Fig. 2(a). As expected, the resistance decreased with the presence of cells in the media. This was clearly observed in the 100 mHz to 100 kHz range (Fig. 2(b)). The viruses exhibited a clustering of negatively charged particles with a large surface area that enabled holding of more negative surface charge [5, 6]; thus, the impedance varied accordingly. Therefore, presence of cells in the suspension improved the conductivity of the suspension at a specific frequency range. On the other hand, the capacitance of the cell suspension was observed to be higher than the media alone (Fig. 2(c)). This was expected from our earlier observations that cells exhibit dielectric characteristics and the effective capacitance is composed of the parallel combination of the media and cells [5, 6].

![Graphs showing electrical results](image-url)

**FIGURE 2:** Electrical characterization of the media and cell samples. (a) Charging profile over time for the cell-free media (Media), cells alone (Control), and virus-expressing cells (WT). (b) Impedance real part versus frequency measurements. (c) Capacitance versus frequency measurements. The cell concentration used was the same in all the samples tested.
Nevertheless, there was little deviation between the control and virus-producing cell capacitances (Fig. 2(c)). Therefore, we examined their corresponding current versus voltage (IV) curves for further investigation. The IV and capacitance-voltage (CV) values were measured for not only the media alone, control cells, and cells expressing the WT virus, but also for cells expressing two different genetic mutants of the WT virus, Mutants A and B. As mentioned earlier, these mutations abrogated the ability of the viral genome to produce viral proteins for virion formation despite the presence of the viral DNA. To conduct the IV measurements, the voltage was swept from -0.5 to +0.5 volts and the subsequent data was plotted on the x-axis, while the current (in the logarithmic scale) was plotted on the y-axis (Fig. 3(a)). The resulting IV curves exhibited a nonlinear relationship, revealing a barrier-like junction (Fig. 3(a)). This junction represents the potential energy of electrons accumulated at the intersection of the metal with the semiconductor material (which in our case is the media in which the cells are suspended in). The minimum peak of this junction is referred to as the “turn-on” voltage, which is highly dependent upon the intrinsic conduction properties of the cell suspension. As can be seen, this peak showed a progressively higher turn-on voltage as the samples moved from media, to control cells, to WT virus-producing cells (Fig. 3(a)). These findings corroborate well with the data presented in Fig. 2, which demonstrated lower resistance values for the WT virus-producing cells, control non-virus producing cells, and media alone, respectively. Therefore, one can observe that the higher the resistance, the lower the corresponding current. Interestingly, the cells harboring the two mutant DNAs that could not produce virus exhibited the highest turn-on voltage and almost the same behavior from -0.25 volt onwards. However, the WT virus-producing cells exhibited lower turn-on voltages compared to cells harboring the mutant DNAs.

To confirm this electrical behavior, the capacitance versus voltage (CV) measurements were conducted in the same manner as IV. The measured capacitance versus DC bias voltage is shown in Fig. 3(b) and one could easily distinguish between the media and the rest of the samples, especially between -0.125 to +0.500 volts. Similar to the IV curves, the CV curves showed progressively higher levels of capacitance due to the presence of cells in the media that could be polarized. The cells expressing the virus particles (WT) could be distinguished from the control cells that did not get any DNA and hence could not produce any virus. Once again, the cells in which the two mutant DNAs were introduced showed a profile that was comparable to each other, yet distinct from that of the cells producing the virus (WT). These results clearly demonstrate that the electrical technique was able to distinguish between cells expressing the virus versus cells that did not express virus. The cells harboring mutant A and B DNA displayed higher capacitive properties than the cells expressing the WT virus. The reason for this observation is not clear since all the three cell lines were prepared at the same time in the same manner and the only difference was the type of DNA that integrated into the genome; Mutant A had a 30 base pair deletion, while Mutant B had a 12 bp deletion only compared to the WT viral genome which has a length of ~ 9875 base pairs [11]. Thus, the genome sizes of the three viruses were essentially the same.

![Figure 3: Further characterization of the electrical properties of the media and cell samples. (a) Measured current-voltage curves. (b) Measured capacitance-voltage curves at 10 Hz. (c) Extracted capacitance-voltage curves at 10 Hz after "de-embedding" the influence of the media. The samples tested were as follows: cell-free media (Media), normal cells alone (Control), wild type virus-expressing cells (WT), Mutant A-expressing cells (Mutant A), and Mutant B-expressing cells (Mutant B). These mutants had two different genetic mutations that abolished the ability of the respective virus to produce viral structural proteins for progeny virion formation despite the presence of the viral genetic material in the cell.](image-url)
C. VALIDATION OF ELECTRICAL RESULTS
To ensure that the mutations that were introduced had actually disabled the ability of the DNA to express viral proteins in Mutants A- and B-expressing stable cell lines, protein extracts were made from control cells with no DNA, cells expressing the WT virus, and cells expressing the two mutant viruses, Mutants A and B. These cell extracts were next tested for the presence of viral structural proteins by western blot analysis using a monoclonal antibody against the viral structural protein Gag (Fig. 4). As can be seen, only the cell extracts that expressed the wild type (WT) virus revealed bands characteristic of viral structural proteins (Pr77Gag and p27Gag), while protein lysates from control cells or the cells containing the mutant viruses did not react with the antibody, revealing no detectable viral structural proteins were produced to generate viral particles.

![Western blot analysis of cells tested in this study: Control, cells not producing virus; WT, wild type virus-producing cells; Mutant A and Mutant B, cells expressing viral DNA with mutation A or mutation B. The anti-p27 MMTV monoclonal antibody was used to detect viral structural proteins Gag, (Pr77Gag and p27Gag), while the anti-β-actin monoclonal antibody was used to detect cellular structural protein actin as a control to show that equal amounts of cell extracts were loaded on the gel.](image)

This is despite the fact that equal amounts of cell lysates were loaded onto the gel (as observed by the similar detection of the ubiquitously expressed cellular protein β-actin). These results confirm that the cells with the mutant viral DNAs indeed did not express the viral structural proteins and therefore could not have generated any virus particles in the cell. Thus, the electrical differences being observed between the wild type and the mutant viruses were due to the intrinsic properties of the mutant cells themselves, i.e., presence or absence of the virus particles since all other variables were the same.

IV. DISCUSSION
The work presented here demonstrates that one can use electrical parameters to characterize cells and furthermore, differentiate between virus-expressing cells from those that do not make virus particles. Cells form the basic unit of life and are primarily composed of water (~70%), while the remaining 30% constitutes a complex mixture of biomolecules that contain proteins, carbohydrates, lipids, nucleic acids, and other small molecules [7].

The data presented in this study show that cells are electrically polarizable entities (Fig. 2) that can be differentiated from virus-producing cells via electrical parameters (Figs. 2 and 3). As expected from the charging profiles of Fig. 2, the dielectric constant of the control and WT virus-producing cell suspension was higher than the media, which was confirmed by the CV measurements (Fig. 3). Thus, it was easier to differentiate between the media, control cells, and WT virus-producing cells in the positively swept voltage range [13]. Therefore, these measurements can be used for detection and identification purposes. As the positive voltage value increased, the capacitance of the control cells increased slightly more than the virus-producing cell capacitance. It is evident that control cells and the surrounding medium have some weak interactions between each other, as the measured CV profile of the control cells was close to the control one with an offset (Fig. 3(c)). On the other hand, the virus-producing cells had slightly stronger interactions with the surrounding medium. The cells harboring the two individual mutant DNAs that abrogated their ability to produce virus particles displayed different capacitive properties than the cells expressing the WT virus (Fig. 3). Furthermore, these two different mutant stable cell lines showed identical electrical profiles (Fig. 3). This result shows that the two types of cells (virus producing and virus non-producing) were different electrically since the only difference between these two cell types was their ability to produce virus particles. These results indicate that the difference in the electrical characteristics of the two cell types was due to their ability to produce virus particles. Interestingly, we expected the virus-producing cells to display a higher capacitive value than the virus-non-producing cell lines since they were not expressing viral proteins and their profile should have been closer to the control cells that also did not produce virus particles.
However, the opposite was observed and the virus-producing WT cells had lower capacitive value. At the onset, the reason for this observation may not be clear since all three cell lines (WT and two mutants) were prepared for electrical characterization at the same time and in the same manner and the only difference was the type of DNA that was introduced into them. Nevertheless, the series capacitance model explains this phenomenon very well. Figure 5 shows the different steps of the mouse mammary tumor virus (MMTV) replication cycle in a cell, the virus used in this study [14]. Briefly, MMTV infects cells using the following steps:

1. First MMTV interacts with a cellular receptor protein on the surface of the target.
2. This allows the virus to fuse with the cell membrane, allowing entry of the viral “capsid” into the cell and its uncoating.
3. The capsid contains the viral genomic RNA (shown in red) and other viral and cellular proteins that help convert the viral RNA into DNA (shown in black), a process called reverse transcription.
4. This DNA (called complementary DNA or cDNA) enters the nucleus and inserts itself into the chromosomes, becoming a gene of the cell.
5. The integrated viral DNA (called proviral DNA) makes viral mRNAs and proteins that are used to make new virus particles in the cytoplasm.
6. The immature virus particles now bud out of the cell, taking part of the cell membrane with them.
7. This is followed by maturation of the virus particles (which is not well-understood), making the virus particles infectious for entry into the next cell.
8. In our experiment, we bypassed the virus infection steps (1-3) and introduced the viral DNA directly into the cells using “electroporation”, a method that temporarily introduces “holes” into the cell membrane, allowing DNA to enter the cell.
9. This DNA now can be “inserted” into the host chromosome just like the viral cDNA, and then the rest of the virus replication cycle can proceed as usual (steps 5-7).

Figure 6 summarizes a set of electrical models that could be used to represent the effective electrical capacitance at a corresponding phase. Figure 6(a) counts for the intrinsic capacitance of the cell itself; Fig. 6(b) represents the effective capacitance of the virus inside the cell scenario within which the virus still does not have any interaction with the cell content. Figure 6(c) models the interaction of viral components inside the cells that are interacting directly with the cell which could result in nonlinear effects, causing a reduction in the capacitance value. Figure 6(d) depicts the assembly of the virus particles inside the infected cell.
As previously mentioned, the capacitance per cell \((C_C)\) is an intrinsic characteristic of the cell. It differs from one cell type to another since it depends on the unique composition of and amounts of cellular components such as lipids, proteins, nucleic acids, etc., specific to each cell type [15]. If these compositions are altered somehow, then the capacitance should change accordingly. Just like the cells, the virus particles also exhibit an intrinsic capacitance \((C_V)\) that is linked directly to its composition [5, 6]. Thus, the effective cell capacitance \((C_{C+} + C_V)\) can be altered by the presence of either complete or parts of a viral particle. Therefore, as presented in Fig. 6(a), initially the effective capacitance of the cell is equal to its intrinsic capacitance. After the expression of the virus particles inside the cell (as in the case of the wild type virus-expressing cell line), if no interaction is assumed between the cell and the virus particles, the effective capacitance is then equal to the sum of both the intrinsic cell and viral capacitances, i.e., \(C_C + C_V\). This is valid as long as the interaction of the virus particle with the cell content is minimal, which may be the case early in the infection cycle as depicted in step 2 of Fig. 5 when only viral proteins are expressed and not the whole virus particles. Once the viral content (genome, RNA, DNA and proteins) becomes a part of the cell composition, nonlinear interactions may occur between the various viral and cellular contents with each other. These nonlinear interactions could cause a reduction in the effective capacitance which could be expressed as a series of two capacitances \(C_C\) and \(C_i\), i.e., \(\left(\frac{C_C}{C_C + C_i}\right)\). The effective capacitance is then dominated by the interaction capacitance \(C_i\). The interaction capacitance also could vary depending upon the phase of the viral production process, as illustrated in steps 3 and 4. The introduction of these interaction capacitances allows one to model the reduction of the capacitance and indeed could be used to identify the virus replication cycle phase. Finally, at late stages of intracellular assembly of virus particles (step 5), the effective capacitance can be expressed as a series model between the intrinsic cell and interaction capacitances summed up with the viral capacitance multiplied by the number of replicated particles. Mathematically, this can be shown as:

\[
C = nC_V + \left[\frac{C_cC_i}{C_c + C_i}\right]
\]

where \(n\) is the number of virus particles assembled inside a cell. Equation (1) describes the effective capacitance that is composed of viral, cell and interaction capacitances to model the presence of viral particles in an immortalized T cell line. This effective value should allow the detection and identification of the virus, depending upon its capacitance value. As follow, the interaction capacitance for the WT sample has been extracted and is shown in Fig. 7. The data presented in Fig. 7 shows that the interaction capacitance is real and can be measured. As can be seen, it was approximately \(~6-7\ \mu F\). The difference in the capacitance of WT and mutant cell capacitance was \(~3 \mu F\) before de-embedding (Fig. 2(b)) and \(~3.5 \mu F\) after de-embedding (Fig. 3(c)), revealing a similar range within two-folds. Since not all cells are undergoing the same stage of virus replication, we may not observe a direct correlation. Overall, based on the previous discussion and the set of models represented by Fig. 6, we feel that this interaction capacitance can be used to even detect and identify different stages of virus cycle within an infected cell.

**FIGURE 6:** Electrical models to explain the reduction in capacitance observed in virus-producing cells compared to virus non-producing cells: (a) intrinsic cell capacitance, (b) the effective capacitance parallel model, (c) the effective capacitance series model, and (d) the effective combined parallel series model. \(C_c\), \(C_p\) and \(C_i\) are the intrinsic cell, virus and interaction capacitance, respectively.

**FIGURE 7:** Extracted interaction capacitance

**V. CONCLUSION**

In summary, based on the work conducted in this study, we present a quick, label-free, novel and sensitive method to differentiate between uninfected and virus-producing cells. The electrical method used is fast and by-passes the need for extraction or extensive processing of biomolecules such as DNA, RNA, or proteins. It is hoped that these observations can eventually lead to the label-free, electrical detection of virally infected cells in biological samples as well as individuals for diagnostic purposes, and for monitoring the different stages of virus or cell replication cycles.

**REFERENCES**


**Farah Mustafa’s research focuses on the molecular basis of diseases induced by retroviruses. Currently, she is studying the mouse mammary tumor virus (MMTV), a retrovirus that causes breast cancer via insertional mutagenesis. Specifically, her laboratory is exploring other mechanisms of tumor induction by MMTV. Their latest work shows that MMTV dysregulates the expression of host microRNAs which have been implicated in many cancers. Currently, they are investigating how this dysregulation at the level of the host can affect virus replication and pathogenesis. Additionally, she is interested in the, regulation of basic steps in MMTV replication and gene expression and designing efficient retroviral-based vectors for human gene therapy.**

**Tahir A. Rizvi’s research interest is directed towards studying molecular steps involved in the replication of retroviruses such as human, simian, and feline immunodeficiency viruses (HIV, SIV & FIV), Mason-Pfizer monkey virus (MPMV), and mouse mammary tumor virus (MMTV) with the ultimate goal of developing retroviral vectors for human gene therapy.**