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Authors: Alireza Alikhani, Milad Gharouni, Hamed Abiri, Fateme Farokhmanesh, Mohammad Abdolahad

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Tracing the pH dependent activation of autophagy in cancer cells by silicon nanowire-based impedance biosensor

Alireza Alikhani[a,b*], Milad Gharouni[a,b*], Hamed Abiri[a,b] , Fateme Farokhmanesh [c], Mohammad Abdolahad[a,b *,#]

[a] Nano Electronic Center of Excellence, Nanobioelectronic Devices Lab, School of Electrical and Computer Eng, University of Tehran, Tehran, Iran, P.O. Box 14395/515, Tehran, Iran

[b] Nano Electronic Center of Excellence, Nanoelectronics Lab, School of Electrical and Computer Eng., University of Tehran, Tehran, Iran, P.O. Box 14395/515, Tehran, Iran

[c] Department of Biotechnoligy, Pasteur Institute of Iran, Tehran, Iran. P.O. Box 1316943551, Tehran, Iran

[#]Email: m.abdolahad@ut.ac.ir

[*] Authors with same collaboration

Highlights

● pH dependent behavior of normal and cancer cells by impedimetric biosensor based on Silicon Nanowires (SiNWs) was introduced

● Autophagy as a biologically activated process in invasive cancer cells, protect them from apoptosis in lower pH

● The measured electrical resistance of MCF10, MCF7 and MDA-MB468 cell lines, by SiNW sensor, in different pH were matched by the biological analyses of their vital functions.

● Just invasive cancer cells exhibited increased electrical resistance in pH 6.5.

Abstract

Monitoring the pH dependent behavior of normal and cancer cells by impedimetric biosensor based on Silicon Nanowires (SiNWs) was introduced to diagnose the invasive cancer cells. Autophagy as a biologically activated process in invasive cancer cells during acidosis, protect them from apoptosis in lower pH which presented in our work. As the autophagy is the only activated pathways which can maintain cellular proliferation in acidic media, responses of SiNW-ECIS in acidified cells could be correlated to the probability of autophagy activation in normal or cancer cells. In contrast, cell survival pathway wasn't activated in low-grade cancer cells which resulted in their acidosis. The measured electrical resistance of MCF10, MCF7, and MDA-MB468 cell lines, by SiNW sensor, in normal and acidic media were matched by the biological analyses of their vital functions. Invasive cancer cells exhibited increased electrical resistance in pH 6.5

meanwhile the two other types of the breast cells exhibited sharp (MCF10) and moderate (MCF7) decrease in their resistance. This procedure would be a new trend in microenvironment based cancer investigation.

Keywords: Impedance sensor; pH; autophagy; silicon nanowire ECIS; cancer cell; acidosis.

1. Introduction

The microenvironmental characteristics of cancer cells could reflect many of their biological parameters to elaborate their phenotype and pH is one of such important specifications[1]. For example, A combination of poor vascular perfusion, regional hypoxia, and increased flux of carbons through fermentative glycolysis leads to extracellular acidosis in the medium of cancer cells with extracellular pH values as low as 6.5 [2]. Notably, environmental acidification is a good indication for cancerous transformation as many pieces of evidence corroborate that the most acidic tumors are more subjected to invade and make local metastasis[3]. The mechanism of such acidification was discussed elsewhere [4]-[5]. Through Pasteur effect, a metabolic switch toward a more glycolytic phenotype, increased reliance on anaerobic metabolism of glucose to lactic acid in cancer cells would occur in cancer cells[6]. Consequently, diffusion limitations and increased production of acid from hypoxic-glycolytic cells, lead to the increase in proton(H^+) concentration within the lumen which causing the interior of the lumen to become highly acidic[7]. Such highly acidic microenvironment protects cancer cells from immune system control and enhances the transition probability from an avascular pre-invasive tumor to an invasive malignancy [8]. Hence, counteracting tumor acidic pH has been considered as a probable therapeutic strategy in clinical settings[9]. Moreover, impedance is frequency depende delectrical resistance which exhibited a strong correlation with the dielectric properties of the material. Two electrodes are connected to

AC voltage in a known range of frequencies during impedance measurement. Hence, the electrical current pass from the first electrode through the second ones. Current would interact with the cells adhered between the electrodes and a fraction of the current would be blocked by the cells depend on their dielectric properties [10]. The reduced current would be translated to increased resistance in electronic language which resulted in an increment of the impedance. Similarly, bio-impedance or biological impedance is defined as the ability of the biological tissue to impede electric current[11]. Any biological perturbations induced on the cells would affect their dielectric properties[12] and subsequently would change the impedance of the sensor. Hence, we can trace the biological effect of the pH changes (as a biological stimulation) by following the changes in the impedance response of the cells. No need to any functionalization and biomarkers, real-time monitoring of the cell vitality states and repeatable response after simple washing protocols are the main advantages of such method. Some limitations such as dependency of the response to the cellular population and just being useful for in vitro applications are disadvantages of this type of sensors. Cellular impedimetric behavior could be equivalent by a combination of capacitor and resistor[13,14]. Also in contrast to normal cells, cancer cells resist against acidic stress by upregulating autophagy as a survival mechanism to maintain their vital functions [9]. Autophagy activates the acidic stress ion channels (ASIC) in cancer cells [15]. So, the progressive state of the tumor exhibited a direct correlation with its resistance to acidic stresses.[16] Due to the above evidences, evaluating the pH of the cell's microenvironment could be lightening for potentially invasive cancer cells. Different approaches were applied to measure the pH of cancer involved medium[17]. In vivo measurement of the tumor pH was carried on by pH-sensitive PET radiotracers, MR spectroscopy and MRI[18]. Such systems are so complicated and expensive which might not be used before the appearance of clinical signatures of the patient and reduce the

prognosis. Also, they can't be used in early diagnosis of cancer which could be achieved by a simple biopsy or pop smear as great in vitro methods.

Here we introduce an electrical biosensing method to monitor the pH dependent behavior of normal and cancer cells by measuring their proliferation dependent electrical resistance in acidic media as one way to diagnose the invasive tumor cells. The proliferation and mitosis rate are our main monitoring parameters. Proliferative behavior of healthy and primary cancer cells in acidic media was compared by cancer cells in normal condition. Probable activation of autophagy was also investigated to elaborate the phenotypic dependent activity of the cells in acidic pH. The electrodes were fabricated by skein SiNWs as a known desired interface for cellular bioelectrical monitoring with 3D interactive surface[19]. which improve signal extraction from the adhered cells[20] [19].Great stable physical and chemical properties in weak acids with moderate pH were another advantages selected the SiNWs as a good candidate for 3D bioelectronics approaches in monitoring the cellular acidification [21][22]. The cells were cultured on the SiNWs covered electrodes for 4h in standard culturing media with normal pH (7.4). Subsequently, the pH of the medium was reduced to known acidic values (6.5&5.5) for a known interval of time. The electrical resistance of cultured cells was extracted up to 24hr after and any correlation between cellular biological states and bioelectrical responses were analyzed. Western blot, Annexin Pi and Zymography were complementary biological assays to elaborate the pH related behavior of the cells. Due to the achieved data, the trend of cells' electrical resistant in acidic pH due to activation of autophagy or acidosis was quantitatively discussed.

2. Material and Methods

2.1. Fabrication process of SiNW impedance sensor

Silicon wafers used as substrates in the present experiment were cleaned through standard RCA#1 cleaning method (NH_4OH:H_2O_2:H_2O solution and volume ratio of 1:1:5 respectively). Subsequently, a thin layer (~ 300 nm) of SiO2 was grown on the substrate by wet oxidation furnace. A 10 nm gold thin layer has been coated on SiO2 by the sputtering system (Veeco Co, USA) at a pressure of 20 m Torr as a catalyst layer. The gold has been patterned and the design of the sensor transferred to the substrate. Au-covered samples were located in a low pressure chemical vapor deposition (LPCVD) system (Roshd e Nano Fanavaran, Iran) with a quartz tube chamber. The growth is a two-step process named as graining and growth. During the graining, a thermal annealing at 450-550◦C for 30 min at the presence of argon (Ar) is carried out which results in the catalyst graining and formation of gold nano-sized islands. During the growth step, a mixture of high purity silane (SiH4) as Si source and Ar as a carrier and dilution gases were introduced to the chamber[23]. Silicon crystalline nanostructures were formed on top of the catalyst islands in the patterned region followed by breaking of the silane to Si and Si-H free radicals.

2.2.Cell culture and seeding

MCF10, MCF7 and MDA-MB468 were obtained from the standard cell banks of national cell bank of Iran (NCBI, Iran). They were isolated from normal sites, grades I and IV of human breast tumors, respectively and were held at 37 °C (5% CO_2, 95% air) in RPMI-1640 medium (Sigma, UK) supplemented with 5% fetal bovine serum (Gibco, USA), and 1% penicillin/streptomycin (Gibco, USA) for MCF7 and MDAMB468 cells and in DMEM-F12(Gibco, USA) supplemented with 10% horse serum(Gibco), 1% antibiotic/antimitotic solution (Gibco, USA), 0.2% NaHCO3(Merck, Germany),insulin (5µg/ml) (Sigma, UK), EGF(10ng/ml)(Sigma, UK) and hydrocortisone(1µg/ml)(Sigma, UK)for MCF10A cells. The fresh medium is replaced every other day. Standard cell culture methods were used for cell propagation. The cells were counted and

suspended in 100 µl of respective culture media for the monoculture experiments before being introduced into the cloning cylinder of the bio impedance sensor. The seeded cells covered about 50% of the surface to let cells have a plenty of space for mitosis. But the pre concentration of dropped normal cells was further because the apoptosis and growth suppression are more probable for normal cell in respect to cancer cells in the similar culturing parameters[19]. So the impedance of normal cells covered sensors would start from higher values (because of further amount of pre dropped cells).We set the cell density at 2000 cells/100 µl for the monoculture experiments.

2.3. MTT ASSAY

The viability of the cells seeded on SiNW surface was experimented by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay [19].After 24 h of incubation, 20 ml of MTT (5 mg/ml) was added to each well. The cells were incubated at 37 C for 3 h. Thereafter, the medium was taken away and the insoluble formazan crystals were dissolved in 200 ml of dimethylsulfoxide (DMSO). The absorbance was measured at 490 nm by Microplate Reader (BioTek, UK). The results were expressed as the percentage of cell growth relative to the control[24].

2.4. Annexin V binding assay

Percentages of apoptotic and necrotic cells were assessed via FACS analysis of Annexin V-FITC and Propidium Iodide (PI)-stained cells. Measurements were carried out using the apoptosis detection kit as suggested by the manufacturer. In brief, the cells were washed with PBS and suspended in 500µL total volume with 490µL binding buffer, 5µL PI and 5µL Annexin V-FITC.

After 15 min incubation in the dark at room temperature, cells were tested for Annexin V binding within 2 h using flow cytometry (EXBio, Czech Republic) [25].

2.5. Western blotting

After the specified treatment, cells were collected and the protein extraction was conducted mainly based on LC3 proteins. At first, the protein concentration was calculated by Bradford's method [26]. Equivalent amounts of protein were boiled for 5 min and detached by SDS-PAGE and were then transferred onto a PVDF membrane (Millipore Corporation, Billerica, MA, USA). The membrane was then blocked with 5% nonfat dry milk in Tris-Buffered-Saline with Tween (TBST) for 1 h at room temperature and incubated with suitable primary antibodies overnight at 4 C. Subsequently, the membrane was washed with TBST and incubated with appropriate secondary antibody for 1 h at room temperature. After three washing steps with TBST, the proteins were observed employing the electrochemical luminescence (ECL) reagent. Analysis of the integrated density of the resultant protein bands was performed by Image J software[27].

2.6. Measurement of MMPs by Zymography

Gelatinase Zymography was performed in 10% NOVEX Pre-Cast SDS Polyacrylamide Gel (Invitrogen Corp, UK) in the presence of 0.1% gelatin under non reducing conditions. Culture media (50 µl) were mixed with sample buffer and loaded for SDS-PAGE with tris glycine SDS buffer as suggested by the manufacturer (Novex, Canada). Samples were not boiled before electrophoresis. Following electrophoresis, the gels were washed twice in 2.5% TritonX-100 for 30 min at room temperature to remove SDS. The gels were then incubated at 37˚C overnight in substrate buffer containing 50 mM Tris-HCl and 10 mM CaCl2 at pH 8.0 and stained with 0.5% Coomassie Blue R250 in 50% methanol and 10% glacial acetic acid for 30 min and de-stained.

Upon denaturation of the enzyme, the gelatinases digest the gelatin in the gel and give clear bands against an intensely stained background. Protein standards were run concurrently and approximate molecular weights were determined by plotting the relative motilities of known proteins[28].

2.7. SEM Methodology

To prepare samples for SEM, after electrical measurement and washing thoroughly by Phosphate buffered slain, PBS (Gibco, USA), the cells were fixed with glutaraldehyde (5%) (Merck, Germany) solution for 5 min followed by drying in the air ambient. The fixed samples were coated by Au (in DC Sputtering system) (NSC, Iran) with the height of 10 nm to increase resolution.

2.8. Impedance measurement

The electrical impedances of normal and malignant cells were measured by NI DAQ USB 6323 connected to the sensors with coaxial wires. The final response was extracted from 10 times measurements in each frequency. To eliminate the effect of medium, the media solution was refreshed before each measurement. (Fig S1)

3. Results and Discussions

3.1. ANVPI analysis to investigate pH dependent behavior in normal and cancer cells

To investigate the effect of reduced pH in probable acidosis of primary cancer cells, we maintained individual samples of MCF10A, MCF7 and MDAMB468 cells in pH 5.5 and 6.5 for 4hrs and subsequently incubated them in normal pH (7.4) for 24hrs. ANPI assays then were experimented to evaluate the pH induced cells apoptosis by comparing with the CTRL cells (Fig 1-A1). ANPI results revealed the increased population of the MCF10 cells entered to both early and late apoptosis by 24hrs maintaining in pH=6.5 (Fig 1-A2 *vs.* Fig 1-A3). Also, the fraction of apoptotic cells was further in pH 5.5 (Fig 1-A4). It is worth noting that the ratio of apoptotic cells, observed

in 12th hr, increased about 30% and 50% in 24th hr for the cells incubated at pH 6.5 and 5.5 respectively.

The ANPI results of MCF7 cells (fig1-B1) revealed the meaningful decrease in the fraction of live cells after maintaining in pH6.5 (fig 1-B2 vs. 2-B3). Also, the cells had been in early apoptotic entered to late apoptotic and subsequently, the late apoptotic cells entered to necrotic state from 12th hrs. to 24th hrs. In pH 5.5 (Fig 1-B4), MCF7 cells severely were affected by acidosis and significant increase in the fraction of apoptotic cells are noticeable after 12hr. The comparative ANPI results taken from MDA-MB cells (Fig 1-C1) presented a minor decrease in the population of live cells 12 hr. and 24 hr. after being acidified in the culture media with pH 6.5 (Fig 1-C2 *vs.* Fig 1-C3). As presented in figure 1-C1, the measured decreased fraction in live cells from 12th hr. to 24th hr. was equal to the minor increment in early apoptotic cells. Also, the reduced fraction of late apoptotic cells revealed no significant entrance of early apoptotic cells to the late apoptotic stage. These results indicated that the MDAMB cells had been cultured in pH 6.5, maintained their vitality by activating the autophagy. Autophagy might be the main function play the key role in different behavior of MDAMB468 invasive cancer cells from other cells in pH 6.5. Increase in apoptotic index of the MDAMB cells had been incubated in pH5.5 (fig 1-C4), indicated that the cells couldn't protect themselves from acidosis in lower pH (~ <6.5) and some secondary phenomena such as autophagy based self-eating would be activated in these pH.

Optical microscopy images are taken from the MCF-7 (Fig2 A-C) in 16th hr. showed a trace of formed lysosomes in acidified MCF7 cells. The presence of lysosome in the cell as the indication of activated autophagy could be observed in MDA-MB468 cells (Fig3 I-K). Moreover, the similar images taken from the MDA-MB468 cells in pH 5.5 showed the entrance of lysosome to form Autolysosome in the cells which activated self-eating mechanism in autophagy and induced

apoptosis in the cell. FESEM micrographs taken from the cells cultured on Silicon nanowire (SiNW) arrays (as culturing substrate) would better elaborate the presence of lysosome beneath or into the cells in different steps of acidosis or autophagy in MCF7 (Fig 2 D-H) and MDA-MB468 (Fig 2 L-P) cells. Also, the 3D interactive surface between cells and NWs could be observed.

3.2 Western blot tans Zymography analyses of acidified cells

We investigated the expression of LC3 associated proteins, (as the major pH dependent protein play the crucial role in autophagy [29] in both MCF7 (Fig 3-A) and MDA-MB468 (Fig 3-B) cells. The result of western blot corroborated the expression of LC3 associated proteins. But the interesting point was that the level of LC3 in MCF7 cells maintained in pH6.5 was more than the suitable range for autophagy of this type of cells. This indicated the acidosis of such cells. in contrast, this level in MDAMB cells with similar maintaining parameters was beneath the suitable level relative to beta-actin in this cells for activation of autophagy in [30].This would support the role of autophagy in maintaining the vitality of metastatic cells in moderate acidic ambient in less than 12hrs. No trace of LC3 was observed in MCF10 cells (not shown here). To ensure if the metastatic cells maintained their invasive behavior in acidic pH we conducted MMP based zymography on the MDA cells had been maintained in pH 6.5 for 4hrs (fig 3-C). Matrix metalloproteinase (MMPs) has an important role in metastasis[31]. The results of zymography indicated the reduced expression of MMP in acidic pH which revealed the decrease in their invasive ability during acidification.

3.3. PH dependent electrical impedance of the cells measured by SiNW-ECIS

Impedance is frequency depended electrical resistance which exhibited a strong correlation with the dielectric properties of the material (Fig S4-1). Such biological behavior could be translated

by combination of capacitor and resistor elements in electronic language if the interfacial electrode was biocompatible (Fig S3). So the ability of the cells in blocking the incoming current from the electrodes in each frequency would be related to their impedance so the electrical capability of the wires would be helpful in live signal extraction from the acidified cells. (Fig 4-I, Fig S2)

Figure 4-II-A presented the changes in mean electrical resistance of MCF-7 cells cultured on SINWs electrodes (measured at f=4 kHz) in normal and acidic pH (6.5, 5.5) in 12^{th} and 24^{th} hrs. vs. 4^{th} hrs. of culturing time. Resistance increment in CTRL cells corroborated their progressed proliferative behavior meanwhile reduction in the resistance of the acidified cells exhibited a strong correlation with pH dependent acidosis and loses in the vital behavior of MCF-7 cells. We assumed the electrical resistance of the cells' layer incubated in normal pH (covered on the electrodes) in 4th hrs. After the start of culturing as normal reference resistance. In normal pH, in the first 8hr, the cells showed a minor increase in their resistance because of the proliferation of cancer cells (Fig4 II-A). In next intervals of time, the resistance continued the increasing regime due to the growth and mitosis of the cells which increased the dielectric media covered on the electrodes. In pH=6.5, the MCF-7 cells were affected by the lower pH of the medium. So they exhibited 30% to 50 % reduction in electrical resistance in 12^{th} and 24^{th} hr. such reduction reached to about 70% and 90% when the cells incubated at pH 5.5. This means that the acidic pH activated the apoptotic pathways on these cells.

The impedance of MDA-MB468 cells was also increased in CTRL pH (Fig 4 II-A) acidic media with pH 6.5 couldn't reduce the impedance of MDAMB cells in first 12hrs which corroborated the activation of autophagy in such cells. The reduction of the impedance of the cells started from 12^{th} hrs. was a corroboration for activation of apoptotic pathways. Also, severe reduction of the mean impedance in MDAMB cells incubated in pH 5.5 could be related to the activation of self-eating

in such cells in acidic media. Similarly, the capacitive behavior of the sensor in the mentioned pH was determined and presented in figure 4 II-B again the different capacitance changes in MDA-MB468 cells maintained in pH 6.5 were recorded with respect to two other types of the cells.

Graphical draw presented in figure 5, clarified the mechanism of autophagy in maintaining the viability of metastatic cells during being exposed to acidic stress. In acidic media with higher pH (6.5), Autolysosome produced by Autophagosome connected to the cells and meanwhile, some metastatic function might be lost, the cells being survived in acidic media. In lower pH (5.5), the entrance of Autolysosome into the cells resulted in cell self-eating and death induced by the autophagy activation. So the function of autophagy in surviving or killing the cell in acidic media is pH dependent.

3.4 Detection limit

Figure6 presented the detection limit profiles of SiNW-ECIS experimented for all three types of assayed breast cells. It could be observed that at least 4hrs are required to observe the electrical response of the sensor after attachment of the cells to the electrodes. Moreover, the pH dependent distinguished response of deicing was recordable in 12th hr (8 hr after attachment) in which the slope of the sensitivity profile became changed in acidic pH for all three type of the cells.

Figure 6 revealed the differential changes in blocking current by cells ($\Delta I/I0$) related to time. Due to differences in cells dialectical features, ($\Delta I/I0$) is directly related to $\Delta_{impedance}$ and based on figure S3-II in every time added normalized impedance in percent. The slope of the equation in every sample revealed the ratio of current blocking and in the simple present ratio of cell's growth. MDA-MB 468 in the low acidic media (pH 6.5) has a positive slope which is revealed the impedance in this condition is increasingly but this slope is lower than Control sample. Time

evolution after spreading stages of the cultured cells, resulted in progressed cellular proliferation [19] which would increase the current blocking ability due to increased filling factor of the surface by enhanced cellular layer . This would enhance the absolute value of ΔI/I0 as presented. Also, we tested about 2×10^4 cells in each assay but the dynamic range of the sensor could be increased to about 10^5 cells in which the surface of the device got saturated. Hence, due to the mitotic rate of cancer cells, preconcentration of about 2×10^4 and monitoring the responses for 72hr could result in non-saturated and desired achievements on the effect of the pH.

4. Conclusion

In summary, autophagy as a protector pathway was activated in MDAMB468 cell lines in pH 6.5 and suppress from their early apoptosis during 12hr. due to the established application of SiNW-ECIS in monitoring the proliferation of the cells, we applied such devices to investigate autophagy activation in acidified normal and cancer cells by monitoring any perturbations induced in cells' proliferation. Metastatic cancer cells continued their proliferation in acidic media detected by their increased impedance recorded by SiNW-ECIS. Western blot and biomolecular assays revealed the activation of autophagy assisted proteins in such cells. In contrast, normal and nonmalignant cancer cells lost their proliferative impedance in even moderate acidic media as recorded by the sensor. Biochemical assays also corroborated the entrance of the cells into apoptosis. It could be concluded that microenvironment based stimulation would be follow able on cellular bioelectrical responses.

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Figures and captions

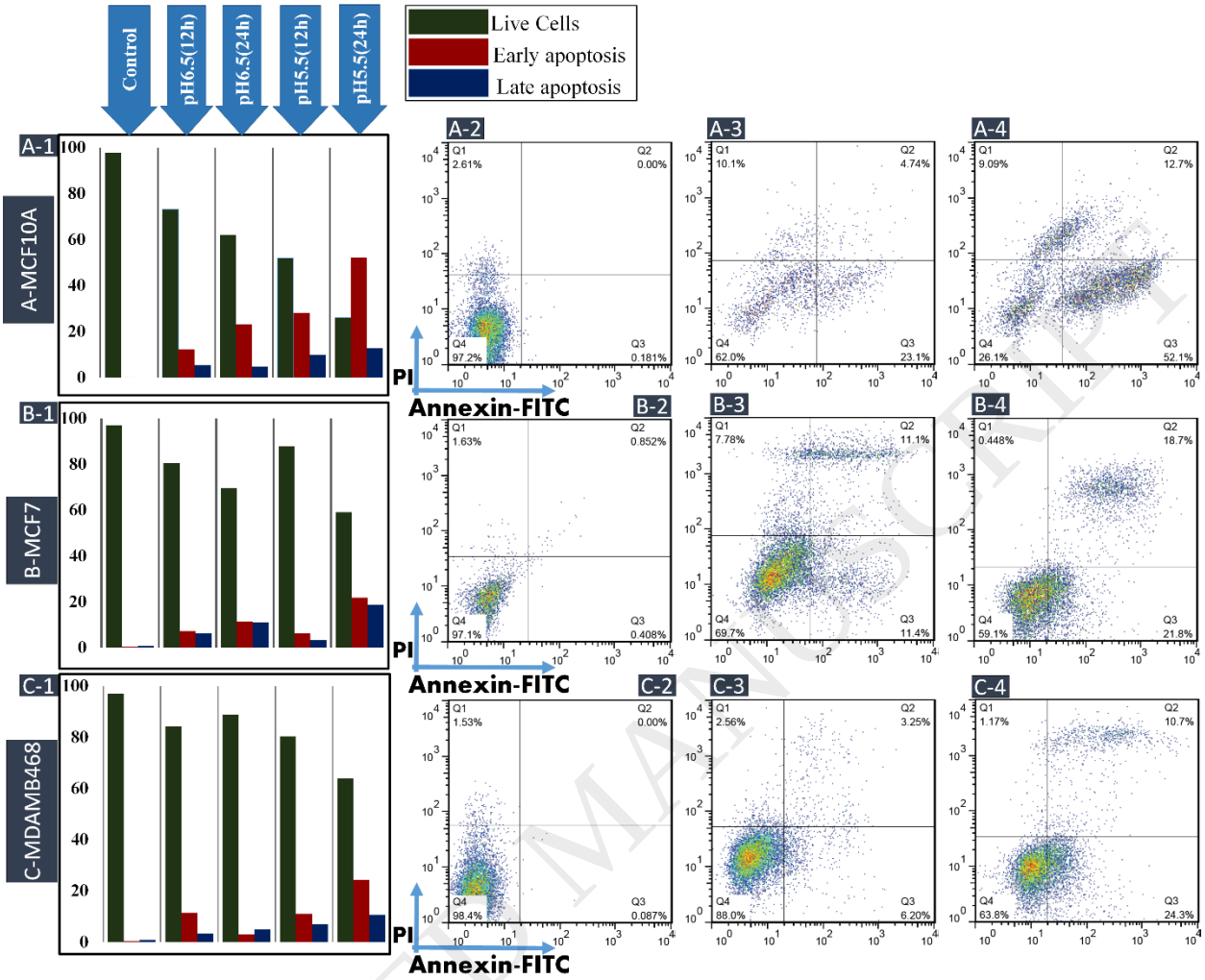

Fig. 1. Annexin PI as a biological assay to detect the percent of the assayed cells in Live, Early apoptotic, late apoptotic and necrotic stages). Comparative mean and raw diagrams of Annexin PI results for: (A) healthy (MCF10), (B) tumorigenic (MCF7), and (C) metastatic (MDA-MB468) breast cell lines in pH: 7.4, 6.5 and 5.5 after 24 hr. entrance of the cells to apoptotic region was suppressed due to their progression in invasive grades. The ANVPI result showed that MCF7 cell line entered to the late apoptosis due to acidic pH (22.5% in early and late apoptosis in pH 6.5 and about 40% in early and late apoptosis in the pH 5.5 after 24th hr.). Although MDA-MB468 resisted against apoptosis in pH 6.5(10% in early and late apoptosis in pH 6.5 and about 35% in early and late apoptosis in the pH 5.5 after 24th hr.).

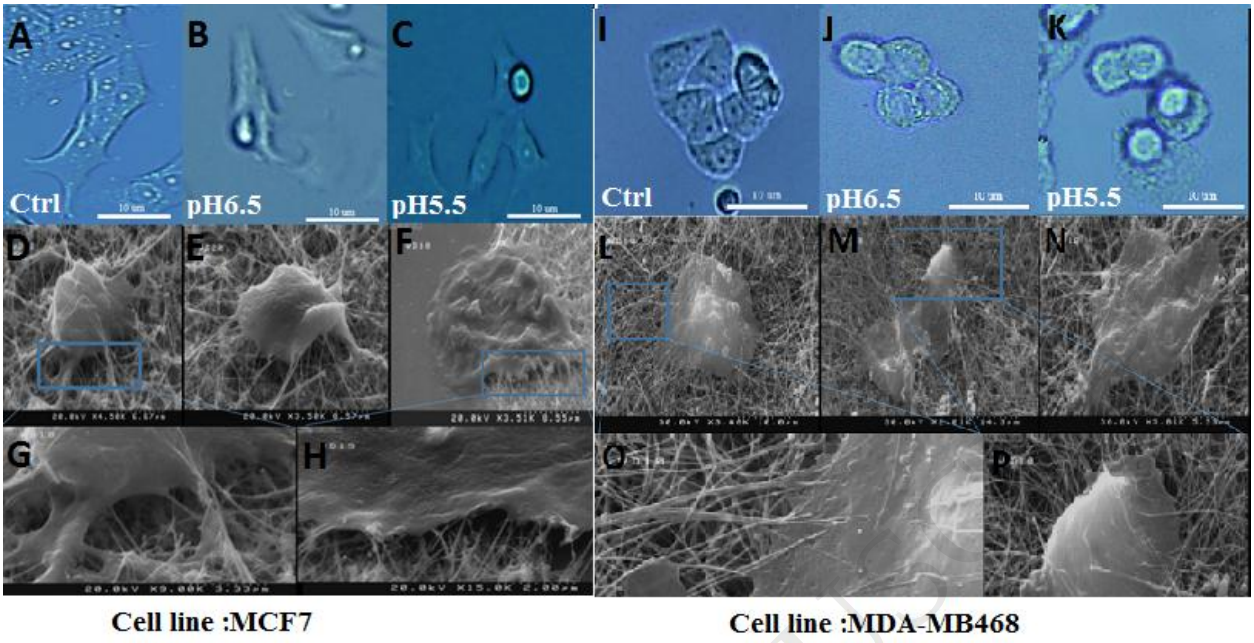

Fig. 2. Optical images of MCF7 (A, B, C) and MDA468 (I, J, K) cells cultured in pH 7.4, 6.5 and 5.5 respectively. Lysosomes play the crucial role in lower pH. Due to activation of autophagy or because of self-eating. FE- SEM images taken 24hr after acidification also show the trace of lysosome in MCF7 in pH: (E) 6.5 and (F) 5.5. In the case of MDA468, in (M) pH 6.5, autophagy suppressed from cell's death and lysosome could be observed beneath the cell. However, in (N) pH 5.5, autophagy induce self-eating and cellular destruction.

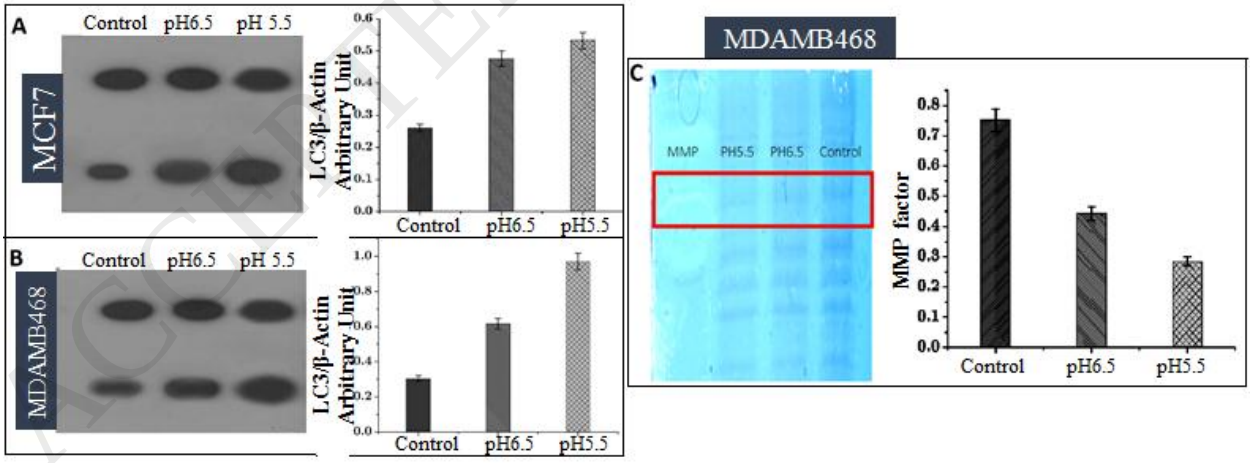

Fig. 3. Western blotting profile of MCF-7 (A) and MDA-MB468 (B) cells incubated in different pH compared based on the expression LC3 associated proteins as an important indication for activation of autophagy. Beta actin was investigated as the reference value. Suitable expression of

LC3 in MDAMB468 cells post cultured in acidic revealed the appropriate activation of autophagy in such cells. Invasive behavior of MDAMB468 cells in acidic pH was also determined by Zymography based on the expression of MMP2 (C) which revealed the reduced metastatic activity of the cells.

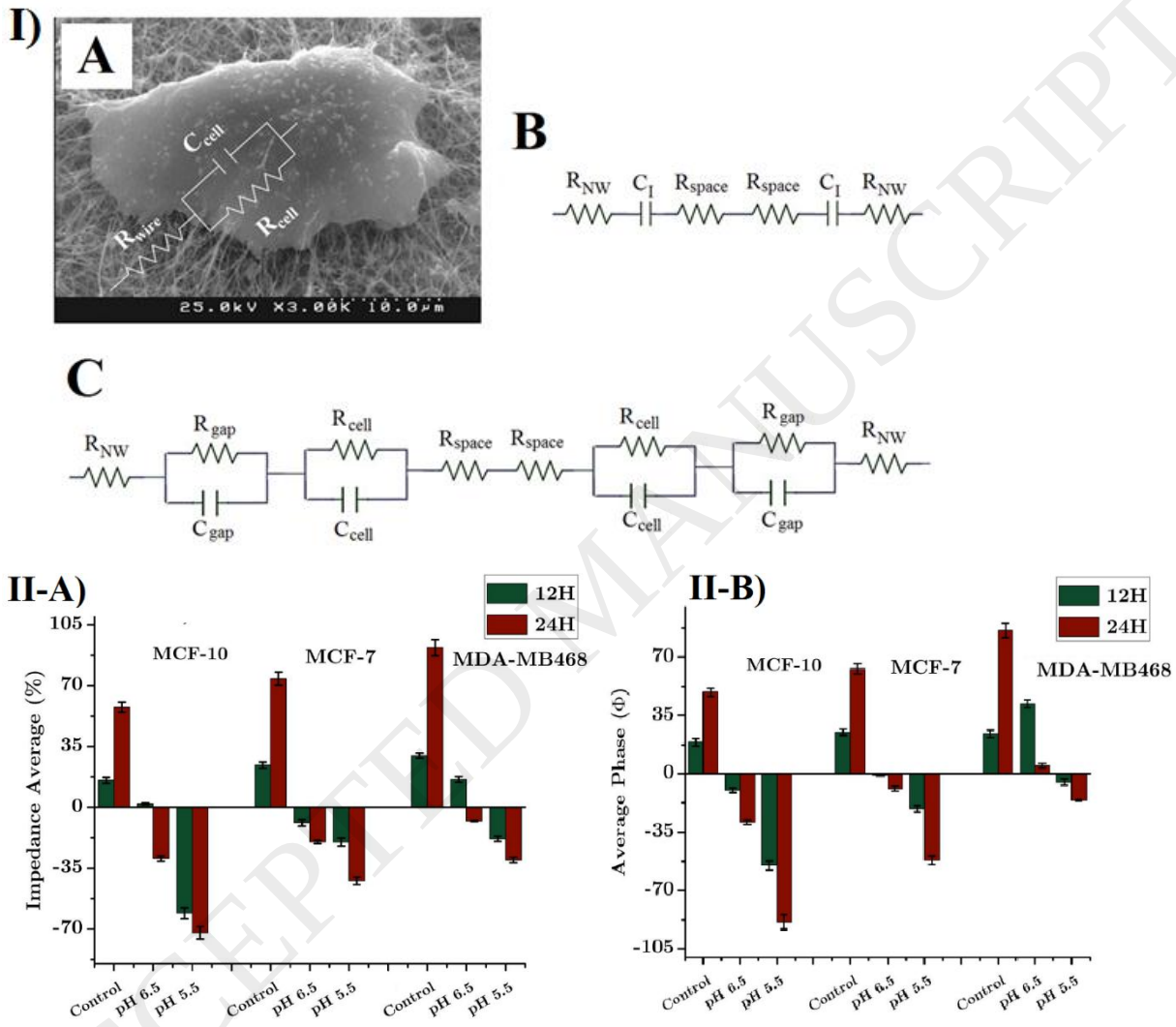

Fig. 4.I) SEM of cell that has been attached on NW with schematic of circuit analyzer (I-A). The current pas through solution (I-B) and equivalent circuit for current pass through cell (I-C). (II) Impedance diagram of the cells cultured on SiNW-ECIS in different pH and various times. The mean diagrams of the changes in cells' resistance (II-A) and capacitance (II-B) after incubation in acidic media were extracted from the raw plots. Just the resistance and capacitance of MDA-MB468 cells exhibited increment in moderate acidic media (pH 6.5) after 12hr.

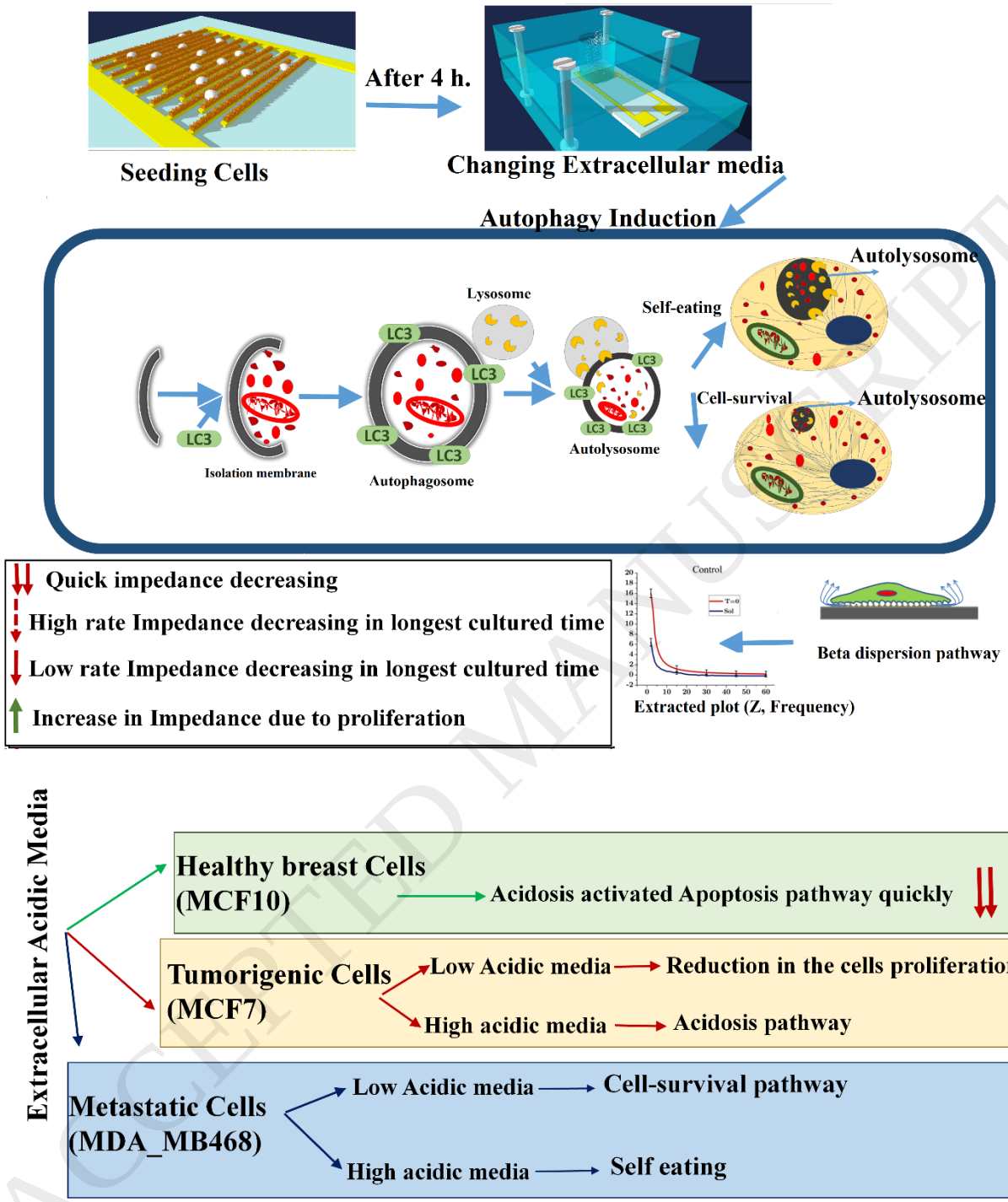

Fig. 5. Graphical draw for the correlation with differential in impedance and normal and cancer cells after acidification. After seeding different types of breast cells on the Si-NW ECIS in the acidified culture media, autophagy induced on cells by expression LC3 associated proteins. Due to expression LC3 and generation Autolysosome, two pathways were activated: Cells survival and

self-eating depend on invasive grade of cancer cell. Each observed biological behavior of cells would translatable to electrical languages by the assistance of beta dispersion phenomena.

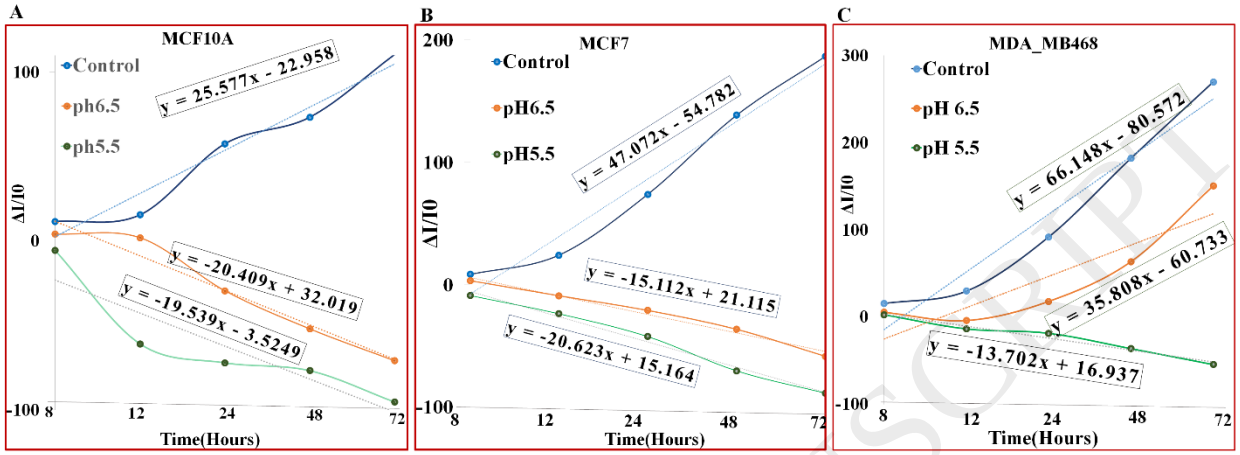

Fig. 6. Detection limit profiles of the SiNW-ECIS in sensing the effect of acidic culture media for MCF10A, MCF7 and MDAMB468 are plotted in A, B and C panels, respectively. The detecting resolution followed the formulated regime is presented in each panel. After 8th hr., all of CTRL cells continued their growth and proliferation observed in the increased current blocking from primary value (delta ΔI/I0). Just malignant cells (MDAMB468) maintained their vitality, observed in their increased ΔI/I0, in weak acidic media.