Critical Reviews™ in Biomedical Engineering, 41(1):37–50 (2013)

Electrical Lysis: Dynamics Revisited and Advances in On-chip Operation

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ABSTRACT: Electrical lysis (EL) is the process of breaking the cell membrane to expose the internal contents under an applied high electric field. Lysis is an important phenomenon for cellular analysis, medical treatment, and biofouling control. This paper aims to review, summarize, and analyze recent advancements on EL. Major databases including PubMed, Ei Engineering Village, IEEE Xplore, and Scholars Portal were searched using relevant keywords. More than 50 articles published in English since 1997 are cited in this article. EL has several key advantages compared to other lysis techniques such as chemical, mechanical, sonication, or laser, including rapid speed of operation, ability to control, miniaturization, low cost, and low power requirement. A variety of cell types have been investigated for including protoplasts, *E. coli*, yeasts, blood cells, and cancer cells. EL has been developed and applied for decontamination, cytology, genetics, single-cell analysis, cancer treatment, and other applications. On-chip EL is a promising technology for multiplexed automated implementation of cell-sample preparation and processing with micro- or nanoliter reagents.

KEY WORDS: cell membrane, electric field, electrical lysis, electroporation, microfluidics, on-chip operation

I. INTRODUCTION

A cell membrane is a complex lipid structure with an intricate mechanism of adhesion and reaction to the topology and chemistry of extracellular space that surrounds the cell and acts as a physical barrier between the intracellular and the extracellular environment. The structure is made of a 4–8 nm–thick phospholipid bilayer mainly composed of phospholipids, which have a hydrophilic head containing a phosphate group and two hydrophobic long tails made of fatty acid chains. Since both intracellular and extracellular mediums are primarily hydrous (containing water) fluids, the hydrophobic tail spontaneously arranges to shield itself from these fluids in a bilayer formation. The cell membrane is comprised of many types of phospholipids including 1,2-dimyristoyl-sn-Glycero-3-phosphorylcholine (DMPC) glycerolipid,[1] ceramides, and sphingolipids, as well as cholesterol, which is essential to regulate the membrane properties. The chemical structure of a DMPC phospholipid molecule and a schematic symbol of a phospholipid comprising a cell membrane are shown in Fig. 1.

In order to analyze the internal contents of cells (such as nucleus, mitochondria, genes, proteins, or other organelles), cells need to be broken open by rupturing the protective cell membrane. This process is known as cell lysis.[2] Several methods are known for cell lysis that use either chemical or physical stimulus.[3,4] Chemical stimulus is primarily done through detergents and is the common laboratory procedure.[5] Other mechanisms for cell lysis include mechanical,[6,7] laser,[8,9] thermal,[10] magnetic,[11] electroosmotic,[12] electrochemical,[13] and electrical lysis.[14–19]

Electrical lysis (EL) refers to breaking open the cell by disintegration of the plasma membrane using a brief, high electric field.[14–19] This phenomenon has also been referred to as electrical breakdown, electrodisruption, or irreversible electroporation.[20–24] The technique of electrical cell lysis, discovered more than 50 years ago, has generated recent research interests due to new microfabrication capabilities that leverage key advantages to on-chip EL, such as rapid operation, low power, low cost, controllability, automation, and on-chip integration. Such advanced microfabrication technologies include

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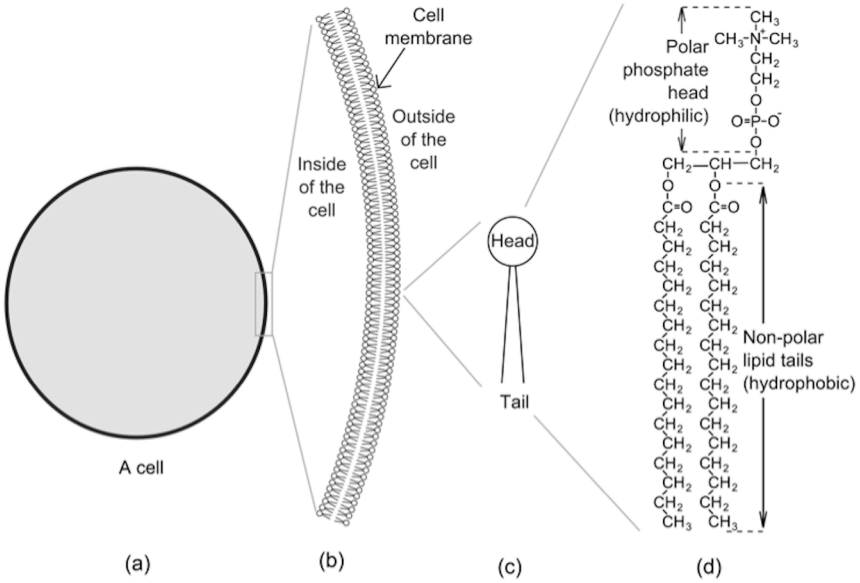

FIGURE 1. Cell membrane composition. (a) A schematic representation of a cell. (b) A cross-sectional view of the cell membrane segment composed of a sheet of phospholipid molecules arranged in a bilayer formation as shown. (c) A schematic symbol of one phospholipid molecule, depicting the head and the tail sections. (d) Chemical composition of one phospholipid molecule (1,2-dimyristoyl-sn-Glycero-3-phosphorylcholine, DMPC), which exists extensively in most cell membranes.

microfluidic device fabrication and microelectromechanical systems (MEMS). Miniature devices could be reliably developed with such fabrication leading to technologies such as lab-on-a-chip (LOC), micro-total-analysis systems (μTAS), and biomedical microelectromechanical systems (bioMEMS). These microfabricated chips miniaturize and integrate macroscale biochemical processes and analysis procedures in microscale or even nanoscale analytics that leads to highly compact, low-cost, and automated systems. On-chip EL could process microliter cell samples inside such microfabricated chips with microchannels or open cavities excited through integrated or external electrodes. In on-chip EL, electrical excitations (DC, AC, or pulsed) rapidly lyse the sample cells on the chip, and the lysate could be analyzed externally, or processed further downstream on the same chip. EL has shown potential to be applied in several biomedical fields, such as food and water decontamination, bioanalysis of intracellular contents, single-cell analysis, and deoxyribonucleic acid (DNA) extraction and analysis, as well as cancer and tumor treatment.[4,25,26] A summary of recent advancements of this research field of recent interest is concisely presented in this article.

II. DYNAMICS OF ELECTRICAL LYSIS

To a first approximation, cells interacting with an external electric field can be modeled using an equivalent RC circuit.[27] The electrical lysis of cells is described with the following expression:[20–22]

$$V = \frac{1.5Ea}{1 + I\omega aC(r_i + r_a/2)} \tag{1}$$

where V is the transmembrane potential, E is the external field strength, a is the radius of the cell, I is the current flow, ω is the frequency of the applied electric field, C is the capacitance of the membranes per unit area, r_i is the resistivity of intracellular electrolyte (cytoplasm), and r_a is the resistivity of extracellular electrolyte.

The phospholipid bilayer membrane of a cell is susceptible to an external electric field through the following two properties:[20,25]

- **Electric dipoles of phospholipids:** The phosphate group (head) is electropositive in nature and the hydrogen chains (tails) are electronegative. Hence, each phospholipid molecule acts as an electric dipole when placed inside an external electric field. These phospholipids undergo reorientation when the electric field is in the MV/m range for many cell lines.
- **Permeability of the membrane:** The bilayer membrane has an electrical permeability that drastically changes with electroporation. In the presence of a high electric field, ions flow through this membrane, resulting in joule heating and thermal phase transition.

Since the generated joule heat during thermal phase transition due to the permeability property of the membrane might damage the contents of the cell, current flow is usually kept very low. The dipole property of phospholipid molecules, on the other hand, plays the dominant role in EL. In addition, bubble formation during the application of the electric pulse due to hydrolysis of water is a major concern for device design and operation.

A. Development of Transmembrane Potential and Formation of Pores

The exact dynamics involved in pore formation within the cell membrane is still a topic of debate.[20,28] One hypothesis states that a high electric field causes an accumulation of charges on both sides of a cell membrane causing reorientation of phospholipid molecules.[20,29] Accordingly, when a cell is suspended in an ionic fluid and is excited by an electric field, charged ions accumulate along the cell membrane, resulting an induced transmembrane poten¬tial across the cell membrane.[20] This transmembrane potential can become higher than 1 V causing electroporation. This transmembrane potential is extrinsic, compared to intrinsic resting action potential that some cells (e.g., living neuron and muscle cells) exhibit as a characteristic spontaneous electrical discharge of 70–90 mV magnitude.[3] The transmembrane potential causes an electrical force on phospholipids, acting as electric dipoles, resulting in a reorientation stress on these phospholipid molecules.[30,31]

If the applied electric field is increased such that the transmembrane potential reaches a critical value of 1 V, the phospholipid molecules undergo conformational changes within the membrane structure. This causes formation of a large number of hydrophilic pores through the cell membrane, especially where the cell membrane surface is perpendicular to the electric field lines.[20] An intermediate state of pores is created that disappear if these pores are not stable. If the pores are stable, they undergo molecular rearrangement to form nanometer-sized pores. The electric field required to initiate pore formation, known as the "threshold electric field," has a typical range of 0.1–0.15 MV/m for erythrocytes, protoplasts, and spheroplasts.[20]

The time interval between the application of the electric field and the initiation of pore formation is termed as the "nucleation period."[32] The pores expand with time if the electric field is not withdrawn.[33] Depending on the duration of the applied electric field, the pores formed in the cell membrane can be reversible, a phenomenon known as electroporation (EP),[34] or become irreversible, a phenomenon known as electrical lysis (EL). EP consists of a cycle of struc¬tural rearrangements of the unidirectional annealing process and unidirectional pore formation process.[33] The time required for the annealing process to be completed is called the "relaxation time" and the transition path difference in pore formation and annealing process is known as electroporation hysteresis.[20,35] The critical pore diameter is ~40 nm for irreversible expansion resulting EL.[31,32,34,36]

B. Dynamics

The sequence of events involved in the EL process can be represented by a state diagram as shown in Fig. 2, or a graph of membrane entropy (ξ) resulting from the reorientation of phospholipids against the elapsed time (t) as shown in Fig. 3, where a few representative temporal paths (or traces) of membrane entropies have been shown as various strengths of electric fields have been applied (red traces with line arrows) or withdrawn (blue traces with solid arrows). Membrane entropy ξ represents the lack of

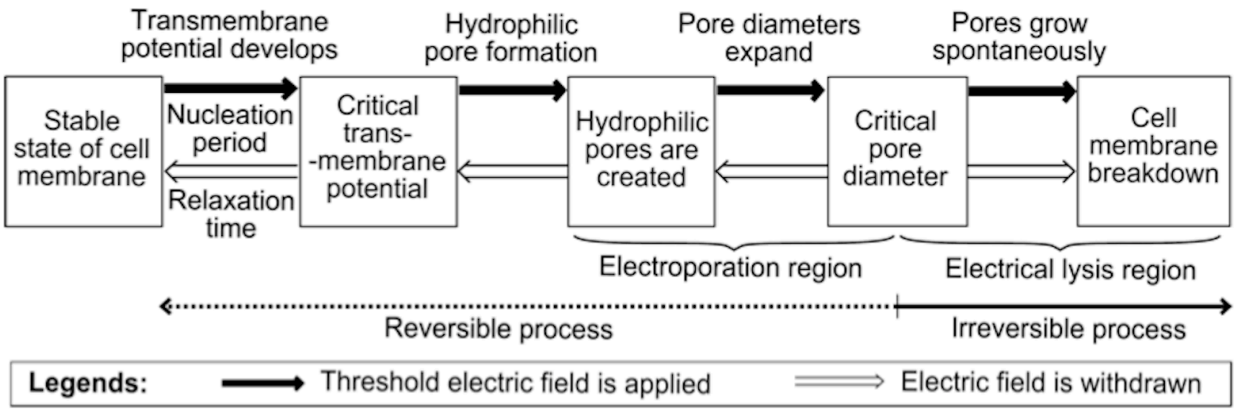

FIGURE 2. A state diagram representing the sequence of events involved in the dynamics of the EL process. Here, the states are represented as rectangles and transitions are indicated using arrows. The solid arrows represent transitions due to application of an electric field above the threshold value. The hollow (white) arrows represent spontaneous transitions after the applied electric field is withdrawn. The process is reversible until the critical pore diameter is reached, after which pores grow spontaneously and an irreversible process is initiated. The region between pore formation and pores attaining critical diameters is denoted as the electroporation region, and the region beyond the critical pore diameter is termed as the electrical lysis region.

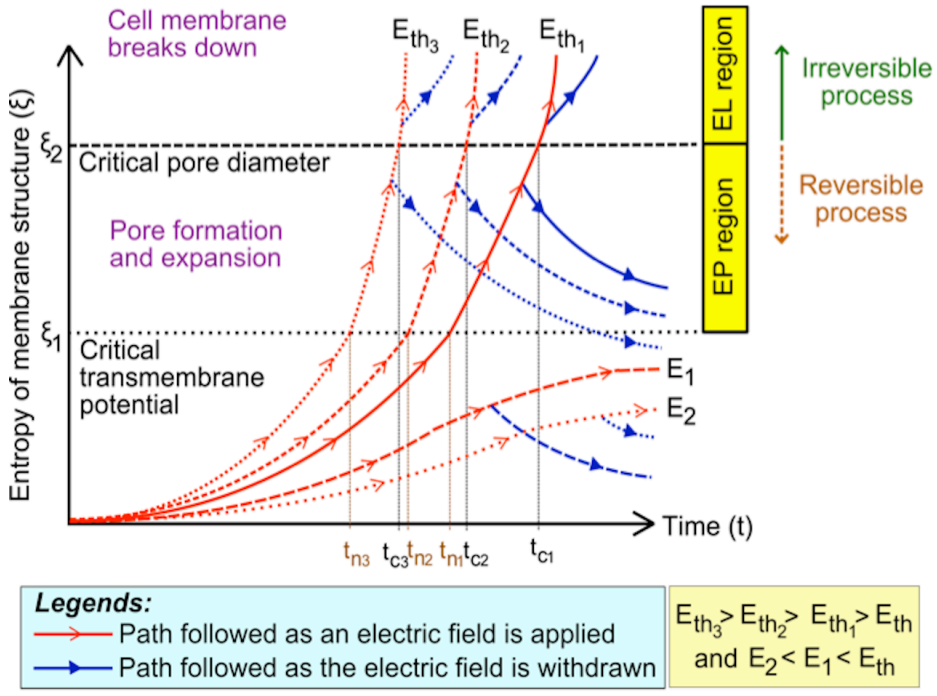

FIGURE 3. Entropy of a cell membrane structure is drawn qualitatively as a function of elapsed time (t). The traces denote entropy change for a range of applied electric fields and entropy change as the electric fields are withdrawn. The nucleation periods are denoted t_{n1}, t_{n2}, t_{n3}. The critical times (e.g., t_{c1}, t_{c2}, t_{c3}) to achieve critical pore diameter reduce with increasing electric fields (i.e., E_{th1}, E_{th2}, E_{th3}) above the threshold electric field (E_{th}). Based on Refs. 20 and 32.

order (i.e., chaos) in the cellular membrane structure, and is primarily responsible for reduced stiffness and nonmonotonic rigidity.[37] In this context, the two figures are simultaneously discussed below to illustrate the relevant dynamics.

1. As an electric field beyond the threshold value is applied on a cell, the stable state of phospholipids within the cell membrane is disrupted and a transmembrane potential develops across the membrane (Fig. 2) that increases the entropy of membrane structural organization (Fig. 3).
2. When this transmembrane potential reaches a critical level, many small hydrophilic pores begin to form. This level of entropy is represented by ξ_1 in Fig. 3. The corresponding times required are nucleation periods (t_{n1}, t_{n2}, t_{n3}). At this entropy, the reorientation force due to the applied threshold electric field (E_{th1}, E_{th2}, E_{th3}) is greater than the orientation force due to hydrophobicity of phospholipids. The formation of these new pores indicate the EP region within the process. If the applied electric field (E_1, E_2) is lower than the threshold value, ξ_1 entropy level would never be reached.
3. If the threshold electric field sustains, the newly formed pores grow in diameter as the entropy increases. When pore diameters reach the critical pore diameter for resealable range, the EP region ends and EL region begins (Figs. 2 and 3). The corresponding entropy is represented by ξ_2 in Fig. 3. The process is reversible up to this level of entropy, after which the process becomes irreversible. The critical time duration (e.g., t_{c1}, t_{c2}, t_{c3}), defined as the time required to reach ξ_2 level of entropy, decreases as the applied electric field (i.e., E_{th1}, E_{th2}, E_{th3}) is increased beyond the threshold electric field.[20]
4. If the applied electric field is withdrawn when the entropy is below ξ_2, then the membrane entropy is decreased and pores reseal leading to the EP phenomenon. If the electric field is withdrawn after the entropy exceeds ξ_2, the membrane entropy continues to increase and pores grow spontaneously, leading to destruction of the cell membrane structural organization resulting cell lysis.[32]

There are a few experimental observations that are difficult to explain with the above dynamics. For example, the membrane poles toward the positive electrode side (the hyperpolarized portion of the cell membrane) were reported to show formation of pores more aggressively compared to the poles toward the negative electrode side (the depolarized membrane).[36]

To explain this aforementioned observation, molecular dynamics representation can be considered, as shown in Fig. 4.[38,39] Here, reorientation of phospholipids within cell membranes due to the threshold electric field is depicted for both hyperpolarized and depolarized membrane. The phospholipids, acting as rigid electric dipoles, are arranged in a stable bilayer cell membrane formation [Fig. 4(a)] and experience reorientation force due to the applied threshold electric field that induces charges (ions) along the cell membrane [Fig. 4(b)].[30,40] The resultant electric force–induced stress attempts to rotate phospholipid molecules and succeeds at locations of faults or weak junctions [Fig. 4(c)]. By noting that only the outer layer of the hyperpolarized membrane and the inner layer of the depolarized membrane would experience the reorientation force [Fig. 4(b). This reorientation of phospholipids is more prominent at the hyperpolarized side of the membrane compared to that at the depolarized side.

III. FACTORS AFFECTING ELECTRICAL LYSIS

A. Pulse Parameters

Electrical excitation parameters greatly influence the lysis operation. These parameters include

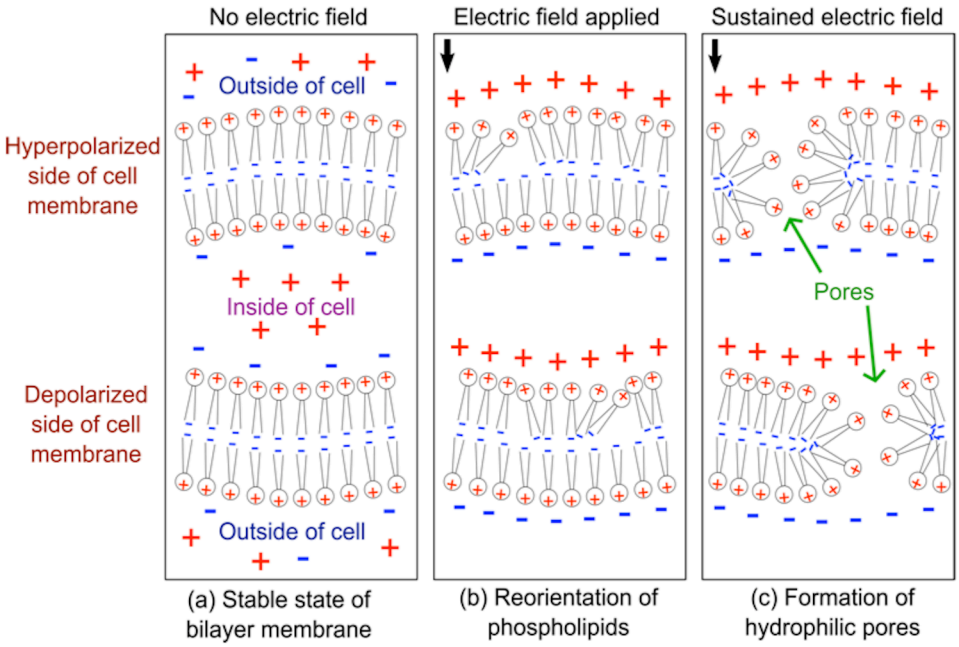

FIGURE 4. Reorientation of phospholipids in hyperpolarized and depolarized sides of a bilayer membrane under a threshold electric field causing pore formation. The arrows in (b) and (c) indicate the directions of the applied electric field. Adapted from Refs. 31, 38, and 40.

the pulse magnitude, pulse duration, number of pulses, frequency, and pulse shape. The ranges of these pulse parameters are critically cell line, cell size, and shape dependent. In this section, some of these parameters are highlighted on the basis of recent findings.

Higher electric fields above a critical value induce EL. For example, using a microfabricated device that had a top and bottom fluidic chamber connected through a 2–4 μm diameter hole (denoted as a microhole) to concentrate electric field around the microhole, EL was reported with 35 V, 100 ms pulses, whereas no EL was observed with 15 V, 100 ms pulses as cells were positioned on top of the microhole, where the microhole is in between two fluidic chamber separated by a membrane.[34] The microhole configuration intensifies the electric field on top of the microhole. In another study, release of intracellular content was observed for *E. coli* with an electric field of >0.1 MV/m; however, at a lower level of electric field, minimal content was released.[18] In addition, the effectiveness of EL has reported to be increased by using multiple pulses instead of one pulse.[18,41]

Survivability of the treated cells with pulsed electric fields has been reported to depend on pulse widths.[26,42] Above the threshold electric field strength, pulses with longer width caused a greater death rate to cells as opposed to shorter pulse widths. For instance, 0.05 MV/m pulses with 6 ms duration were found to be sufficient to lyse Jurkat cells, whereas the cell membranes of the same type of sample were intact with 6 MV/m pulses with 60 ns duration.[43] Cells in the latter case also died because of severe damage to internal organelles. Similar short-width pulses were shown to damage internal organelles that lead to cell death, without rupturing cell membrane. For instance, Leukemia cell membranes were damaged with pulses of 0.14 MV/m and 5 μs width, but no cell membrane damage was observed with pulses of 1.35 MV/m and 50 ns width, even though >90% of cells died within three days after exposure.[22] In addition, increased caspase (enzymes causing programmed cell death or apopto-

sis) activity was observed for longer pulses (300 ns) compared to shorter pulses (10 ns).[44]

B. Lysis Time

The lysis time is critically dependent on the strength of the electric field, is a function of the duration of the electric field. Time between EL initiation and complete disruption was reported to be <25 ms for erythrocytes with excitation pulse of 1 s.[45] Lysis time of <33 ms (within one frame of captured video) is reported for rat basophilic leukemia (RBL) cells with pulses of 1 ms width, whereas lysis time increased to >1 s with pulses of 100 μs width for the same electric field.[36] Time required for lysis of acute myloid leukemia (AML) cells using the EL method is reported to be less than 300 ms after excitation is applied.[46] Through reduction of fluorescence dye, EL of <66 ms was demonstrated for RBL cells.[17]

C. Types of Excitation

Even though pulse excitation is the most common excitation source used for EL, other types of excitations were also reported, such as alternating current (AC) or direct current (DC). EL of several types of protoplasts was reported with 1 MHz AC excitation.[29,47] For the case of AC excitations, cells treated with higher applied voltage and frequency resulted in more cell lysis, whereas cells treated with lower applied voltage and frequency resulted more ghost cells (keratinized denucleated cells).[27] With AC excitations, cells undergo a dielectrophoretic force in addition to the electrical lysis phenomenon. This dielectrophoretic force is responsible for the stretching of cells between electrodes, which might additionally contribute to cell lysis.[29] Recently, EL of vaccinia virus was reported using AC excitation.[19] Most studies for DC excitation consist of a microchannel with a high electric field with a steady rate of cell flow. An example of DC excitation for EL is erythrocytes collected from human blood samples that were lysed during 1 s excitation of 1.4 kV across a 1 cm–long microchannel.[45] Some other examples of DC excitation are AML cells lysed with 1.38 kV across a 4.2 cm–long microchannel in less than 300 ms,[46] Chinese hamster ovary and *E. coli* cells lysed with 0.06 and 0.24 MV/m electric fields, respectively,[14,48] and RBC lysed using 0.12 MV/m electric fields.[15]

D. Buffer Type

EL is expected to depend on buffer type due to differences in conductivity, permeability, osmolarity, and density of ionic molecules. For instance, cells were observed to swell due to the EP phenomena at different rates with various osmolarities of the buffer medium.[48] The average swelling of cells in hypotonic buffer was 175%, in isotonic buffer 155%, and in hypertonic buffer 160% when subjected to 0.05 MV/m electric fields for 300 ms.

E. Cell Type

Chinese cabbage protoplasts and radish protoplasts were lysed with a smaller electric field (0.15 MV/m) compared to yeast (*S. cerevisiae*) protoplast and *E. coli* (1 MV/m); however, the former required longer pulses (1 ms) compared to the latter (100 and 500 μs, respectively).[29] Leukocytes or WBC (1:100 dilution) from blood samples were lysed using 10 μs pulses of 50 MV/m.[30] Chinese hamster ovary, *E. coli,* and RBC were lysed using 0.06, 0.24, and 0.12 MV/m electric fields, respectively, with DC excitation.[14,15,48] This demonstrates that the excitation parameters (such as electric field, pulse width) required for cellular breakdown is largely dependent on the cell type. In addition, cell growth phase (differentiated live cells, dead cells) might have influence on EL.[21]

F. Specialized Geometric Structures of Devices for EL

Channel geometry can be manipulated to amplify the electric field in a certain section of the channel to achieve EL.[14,15,27,30,34] Such modifications allowed developing the threshold electric field with lower than the required applied voltage without geometric modification. Furthermore, such modifications could result in lower joule heating.[14,15] Some examples of modifications are outlined here (Fig. 5).

1. Integrated electrode configurations are critical to define the electric field distribution. Examples of planner

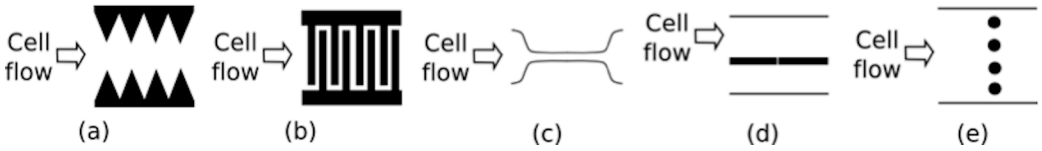

FIGURE 5. Specialized geometric configurations to develop electric fields for on-chip EL. (a) Top view of a sawtooth electrode configuration where cells flow in between the electrodes. (b) Top view of an interdigitated electrode configuration where cells flow above the electrodes. (c) Top view of a wide-narrow microchannel configuration where cells are flown through the narrow microchannel with high electric field. The electrodes are usually in the wide section of the microchannel. (d) Cross-sectional view of a microhole configuration where cells flow thorough the top microchannel. The electric fields are generated with one electrode in the top microchannel, while the other is in the bottom microchannel. (e) Top view of a 3D electrode configuration where the black circles are the cylindrical electrodes.

electrode configuration include sawtooth[15,27,29] and interdigitated.[49,50] The sawtooth configuration generates nonuniform electric field distribution with localized high electric field at the tip of the electrodes, whereas the interdigitated configuration creates relatively uniform electric field distribution.

2. The electric field developed inside the microchannel is directly proportional to microchannel widths for a certain microchannel height.[14] A modification that used this relationship has a narrow (pinched) section of microchannel to create higher electric fields compared to the wider section of the microchannel. Such a device was used to lyse Chinese hamster ovary and *E. coli* cells with 0.06 and 0.24 MV/m electric fields, respectively.[14,48,51] A similar device lysed RBC in continuous flow mode, where a bias voltage of 50 V created 0.12 MV/m in a narrow section of the microchannel that was sufficient for EL.[15] The applied voltages that would have been required without modifications are many times higher. However, such modification only allows cells that are inside the narrow section to be lysed; hence, cells should flow in a single profile through this section of the microchannel.
3. Another geometric modification that concentrates the electric field uses a microhole between two fluidic chambers.[34,52] The diameter of the microhole (2–4 μm diameter) needs to be smaller compared to the cell diameter (~20 μm). When a cell settled on top of the microhole, a voltage pulse was applied to lyse (or electroporate) the cell. Human prostate adenocarcinoma (ND-1) cells were lysed with excitation of only 35 V, 100 ms pulses using such a microdevice, much less than without the microhole.[34] However, this modification requires each cell to be positioned on top of the microchannel before it can be lysed.
4. Instead of planar electrodes, 3D electrodes with thickness similar to the microchannel depth were shown to be more effective for EL.[27,30] The advantage of 3D electrodes includes more volume in the microchannels energized with a certain amount of energy delivery compared to the planar electrodes resulting a higher EL rate. For example, 30% EL was achieved with 3D electrodes compared to 8% with planar electrodes.[30]

IV. SECONDARY EFFECTS OF A HIGH ELECTRIC FIELD

In addition to EL, a high electric field pulse might also affect cells in many other ways. These effects might be important for various fields of biochemistry and molecular biology. As pulse excitation is applied, adjacent cell membranes might fuse together,

a phenomenon known as electrofusion that was observed in several studies.[20,27,33] Initially, it was thought to be the result of thermal melting and merging of cell membranes due to joule heating. However, it is now generally accepted that this phenomenon is due to nonthermal effects related to electric fields below threshold level.[25] In addition, a variety of morphological changes of cell membranes causing deformation were noted after electric pulse treatment, such as membrane extrusion[27] or vesicle formation.[33]

V. APPLICATIONS OF ELECTRICAL LYSIS

On-chip EL, electrically lysing of biological cells on microfabricated chips, has been demonstrated to be applicable for various research fields. In this technique, minute (microliter volume) cell samples are introduced on or inside the chip containing microchannels or an open cavity with integrated or external electrodes. Subsequently, brief electrical excitation is applied that creates localized electric fields beyond the critical strength around the sample cells, and this field is sustained longer than the critical time duration for lysis. Such a process results in breakdown of the cell membrane, and the lysate could be collected for analysis, or further processed in assays downstream on-chip using LOC or μTAS systems. MEMS and microfluidic fabrication technologies enabled researchers to design, develop, and test various fluidic chambers of microliter volume with different configurations of integrated electrodes usually deposited with the MEMS lithography technique. A few examples of these microchannel design and electrode configurations are illustrated in Fig. 5. In general, such microstructures are prepared on glass or polymer (e.g., PDMS, SU8) substrate, while the electrodes are made of inert platinum (Pt) or gold (Au). Integrated electrode layers generated with MEMS-based soft lithography techniques enable generation of the high electric fields needed to lyse cells with a low applied potential and low power consumption. This is critical to allow controlled experimentations with various electrical excitation parameters, to reduce joule heating, that broaden the future potential of practical, *in vivo* applications.

It has been demonstrated that on-chip EL has many advantages over other lysis methods. EL is faster compared to most other lysis techniques except laser-based lysis, but offers simple and low-cost implementation compared to laser-based lysis. Furthermore, controllability of on-chip EL is superior and suitable for automated operation. These advantages render this method more suitable for on-chip implementation for LOC, μTAS, and bioMEMS. Primary application areas with active research interests are listed below.

A. Bioanalysis Device

On-chip EL can be implemented in and integrated within a micro-device to perform bioanalysis of subcellular contents.[27,29] Some examples of application for such EL-based microdevice are cytology, genetics and molecular biology, on-chip sample preparation of respiratory bacterial pathogens, chemical cytometry analysis at the single-cell level, and controlled cell growth–interfering agent.[23,51,53]

B. Biofouling Treatment

EL inducing apoptosis of bacteria and other microorganisms can be an effective tool for controlling biofouling (contamination due to growth of bacteria) by treating food, water, and beverages.[21,41–43]

C. Single-Cell Analysis

Single-cell analysis is feasible with only electroporation and electrical lysis.[34] This type of analysis is significant because it aids in understanding of features that cannot be detected in a colony-based approach. Glutathione (GSH) within individual erythrocytes has been analyzed using the EL method to study the mechanism of various biological processes in the human body.[45] To increase throughput, parallel processing of single-cell analysis using EL followed by capillary an electrophoresis technique was demonstrated through a parallel microchannel–based device.[46]

D. Cancer and Tumor Treatment

Irreversible electroporation for tumor ablation in the application of cancer and tumor treatment by selectively killing unwanted cells by zapping

them with a high electric field is very promising research.[22,54,55]

VI. RECENT ADVANCES OF ON-CHIP ELECTRICAL LYSIS

On-chip electroporation with integrated electrodes offers the advantage of low-voltage, low-power operation, minute sample requirement, controllability, and integration within the on-chip system through advanced technologies including bioMEMS, LOC, and μTAS. Recent study of the EL phenomenon has further enhanced our understanding of the relevant dynamics,[36,38,40,48] and the acquired knowledge is being applied in various scientific areas including biotechnology, molecular biology, and biomedical engineering. Specific applications of such technology include subcellular analysis,[27,29] gene transfection,[30] biofouling treatment,[42] integration of multiple biochemical processing,[45] high-throughput parallel bioassay preparation,[46] cancer treatment,[55] and potential integrated DNA detection on-chip.[56] Advances in microfabrication technologies have facilitated the progression of this research.[26,54,58,59] A chronological list of major findings on EL for the last 15 years is summarized in Table 1.

TABLE 1. Chronological list of the major findings related to on-chip EL within the last decade

Year	Sample	Excitation	Major findings related to EL	Refs.
1997	*E. coli*, *A. salina*	Pulse excitation V_s = 40 kV t_p = 60 ns, 300 ns, 2 μs EF = 0.01–10 MV/m	• Energy required: ↓ for *E. coli* with 60 ns pulses ↑ for *A. salina* with <5 ms pulses	42
1997	Leukemia cells	Pulse excitation. a. t_p = 5 μs, EF = 0.14 MV/m b. t_p = 50 ns, 1.3 MV/m	• Observation: ↑ pulse width damaged cell membranes ↓ pulse width damaged organelle membranes	22
1999	Various protoplasts, *E. coli*	AC and pulse excitations. EF ≤ 2.1 kV/m f_{ac} ≤ 2 MHz. t_p = 100 μs to 1 ms	• Excitation parameters required for EL was dependent on cell types	29
2000	Bacteria cells and yeasts	Various excitations EF = 0.2–10 MV/m t_p = 10 ns to 300 μs	• Factors influencing EL include media, growth phase, cell type, field distribution, pulse type, pulse duration, and electric field strength	21
2001	Prostate adenocarcinoma	Pulse excitation V_s = 15 and 35 V t_p = 100 ms	• Observations with cell on top of microhole: 35 V→EL	34
2002	Human Jurkat cells	Pulse excitation t_p = 10, 60, 300 ns EF = 2.6–30 MV/m	• Pulse treatment killed over 90% of cells • ↑ pulse width, then ↑ caspase activity	44
2003	Rat basophilic leukemia	Pulse excitation V_s = 10–40 V t_p = 100–1000 μs	• Faster dye loss from cells with: ↑ magnitude of pulse, ↑ pulse width, ↓ E_{gap}	36
2004	Jurkat cells	Pulse excitation t_p = 10 ns EF = 30 MV/m	• Induced apoptosis of cells • Pores resealed (no lysis)	43
2004	Erythrocytes	DC excitation V_s = 1.4 kV	• Cells were lysed • Analyzed for glutathione	45
2004	Leukemia cells	DC excitation V_s = 1.38 kV	• Cell membrane was disrupted • Nucleus was not disrupted	46

TABLE 1. Continued

Year	Sample	Excitation	Major findings related to EL	Refs.
2004	*O. proteus, L. brevis, Z. bailii*	Pulse excitation E_{gap} = 1 cm V_s < 29 kV t_p = 2 or 3 μs EF = 2.5–3 MV/m	• ↑ number of pulses, then ↑ inactivation • Monopolar pulses were superior to bipolar • Increased susceptibility in alcoholic beverages	41
2005	Human colon Carcinoma	AC excitation E_{gap} = 30 μm a. V_{pp} = 6V, f_{ac} = 5 kHz b. V_{pp} = 8.5V, f_{ac} = 10 kHz	Lysis (%): a. 28 b. 7 Ghost cells (%): a. 81 b. 7	27
2005	Human leukemic cells	DC excitation All chambers in series I = 2 mA, t_p = 10 min	Chamber / Damaged cells (%) / Lysed cells (%) Anodic: 55 40 Cathodic: 10 70 Intermediate: 15 10	53
2006	Leukocytes	Pulse excitation. V_s = 10 V E_{gap} = 20 μm t_p = 100 μs. f_p = 4 Hz	• Observations: 1. Electroporation and large pore formation 2. 30% of the cells lysed	30
2006	Chinese hamster ovary	DC excitation Various buffers EF = 0.02–0.06 MV/m	• Cell swelling observed, but no strong correlation with cell size • EL within 150 ms with EF > 0.06 MV/m	48
2006	Chinese hamster ovary	DC excitation EF > 0.06 MV/m Pinched μch	• Increased EF reduced time required for EL	51
2006	*E. coli*	DC excitation V_s ≤ 1.5 kV Pinched μch	• EF < 30 kV/m→cell viability not affected • EF > 200 kV/m→cell viability decreased	14
2007	Red blood cells	DC excitation V_s = 30–80 V Pinched μch	• 30 V→85% of the cells reached outlet port. • > 50 V→no cells reached the outlet port	15
2007	Human breast cancer cells	Pulse excitation V_s = 40 V t_p = 10 μs	• Messenger ribonucleic acid (mRNA) analysis: - achieved at single-cell (spheroids) level - required stronger pulses than cultured cells	16
2007	Vaccinia virus	AC excitation V_{pp} = 20 V f_{ac} = 100 kHz	• Observation of fragments of cell structures after EL with electron microscope	19
2007	Plant protoplasts	AC excitation Pinched μch f_{ac} = 5–1000 kHz	• EL with 10 V or less at a frequency of 1 MHz • ↓ breakdown voltage, as ↑ cell size	47
2008	Rat basophilic leukemia	Pulse excitation V_s = 100 V t_p = 10 ms E_{gap} = ~1 mm	• EL detected within 66 ms • Electrophoretic separation of cytoplasm was demonstrated	17
2008	*B. pertussis*	Pulse excitation. V_s = 300 V t_p = 50 μs	• Observation of cell debris after treatment with electron microscope • Released DNA was viable	23
2008	*E. coli*	Pulse excitation. t_p = 3 s EF = 50–125 kV/m	• EL released subcellular contents • Minimal dye release below 100 kV/m	18
2010	*E. coli*	AC excitation V_{RMS} = 240–280 V	• RNA collected from lysed bacteria cells	39

TABLE 1. Continued

Year	Sample	Excitation	Major findings related to EL	Refs.
2010	*Yeast*	AC excitation. V_{pp} = 55 V. f = 50 kHz.	• Up to 99% lysis efficiency demonstrated	59
2010	*Erythrocyte, Leukemia cells*	DC-biased AC excitation V_{dc} = 15–160 V V_{ac} = 0–145 VRMS	• By controlling DC and AC component, simultaneous trapping, flow control and lysis were achieved	60
2011	*Erythrocyte*	Narrow channel, EF = 1.1 to 1.2 kV/cm	• Average time required for lysis was 92 ms for normal cells	61
2012	*Yeast*	AC excitation V_{pp} = 52–130 V f = 10 kHz	• 600 µL/min throughput • Achieved up to 90% lysis efficiency	62
2012	*Chinese hamster ovary, Salmonella typhimurium*	Pulse excitation. EF = 1.8–2 kV/cm t_p = 100 ms N = 10	• Extraction of genomic DNA • Extraction efficiency increased from 5 ± 0.6% to 45 ± 10% with reduced concentration from 10^6 to 10^2 CFU/mL for bacterial cells • Electric field required for lysis of bacterial cells and mammalian cells were similar	63

Abbreviations/symbols—EF: electric field. EL: electrical lysis. MFD: microfluidic device. µch: microchannel. DC: direct current. AC: alternating current. E_{gap}: electrode gap. f_{ac}: frequency. f_p: pulse rate. I: current flow. N: number of pulses. t_p: pulse width. V_s: supply voltage. V_{pp}: pick-to-pick supply voltage. ↑: increase. ↓: decrease. →: leads to.

VII. CONCLUSIONS

With the ability of advanced fabrication technologies, EL has emerged as a strong candidate for LOC, bioMEMS, and µTAS enabling the technology of cell lysis. EL is a rapid, low cost, nonresidue, on-chip implementable, and highly controllable method that can be easily integrated with other microfluidic components. This concept has enabled cell lysis to be applicable in micro- and nanoliter scale. EL has many potential uses in various biomedical applications, including bacterial decontamination, microdevices for cytology and genetics, and single-cell analysis, as well as cancer and tumor treatments.[64] However, optimized designs and applications of on-chip EL integrated with other cell sample–preparing protocols in an automated system require further understanding of the phenomenon, stratification of the control parameters, and resolving sustained issues.

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