Electroporators Based on Digital Formation of Arbitrarily-Shaped Electroporation Pulses

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We have designed and constructed a Digital Poration System (DPS), a versatile device for electrotreatment of biological objects by electric field pulses. The feature distinguishing DPS from the currently available electroporators that are based on capacitor discharge through the load is the use of a digital-to-analog converter card as the pulse generator, with further amplification of the pulse by both voltage and current. The pulse shape is arbitrarily programmable in DPS and includes bipolar pulses, unlike other electroporators, which provide only unipolar exponentially decaying and rectangular pulses. In DPS, many of the drawbacks inherent in other electrop-orators have been eliminated, including the need for additional external pulse analyzer (optimizer) monitoring and logging the electroporation processes, the need to recharge the capacitor before any new pulse, poor precision in setting and measuring the pulse parameters, the need for an additional generator of a long-lasting low-voltage signal for electrophoresis of ions into the porated object, the need for additional AC generators for alignment of cells before, after, and during electroporation, and the need for an additional microchip for control of multipulse and/or repetitive protocols. The slew rate of about 1 V/ns, which is required for electroporation of most somatic cells has been achieved in DPS, for ±250 V output voltage and 500 Ω load resistance. The application area of DPS is much wider than that of other available porators and includes electrochemotherapy, cell electrofusion, oocyte activation by calcium wave mimicking, dielectrophoretic bunching and alignment of cells, sorting of cells, and electrophoresis of charged species into the cells.

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Electroporation (EP) is becoming the foremost method of transient and temporary pore formation in cell membranes.[1,2] EP has become an essential component in biotechnology methods,[3–5] as well as in fundamental research including biophysics, molecular biology, genetics, and developmental biology,[6–8] while the tasks of EP range from cell fusion and sorting to drug or gene delivery into the cell.[9]

More than half of EP/electrofusion papers published in Medline-cited journals were published after 2001, indicating a burst in the use of this technique; it is currently a common alternative to physical treatment of cells such as microinjection[10,11] or ultrasound,[9] chemical treatment such as calcium phosphate co-precipitation[12] or incubation with ionophore,[13] and even adenoviral transfer.[14] Apart from EP and cell electrofusion, electrotreatment of biological cells causes several interrelated cellular level phenomena[15,16,17] including: dielectrophoretic bunching of cells or alignment of cells into pearl chains; orientational alignment of asymmetrical cells; orientational alignment of symmetrical but dielectrically inhomogeneous cells caused by interaction of the external field with polarization charges induced by the same external field; introduction or removal of molecules and ions during the pore lifetime; electrophoresis of charged species in the external electric field; cell separation and selection; cell electrorotation.

The inherent advantages of EP are ease of operation, higher degree of control and higher measurement accuracy of electric pulse parameters compared to parameters of other procedures, and thus higher ability to optimize the EP protocols compared with protocols of other cell treatment types. Another advantage of EP is much smaller toxicity compared with the above-mentioned chemical methods of cell permeabilization and fusion.

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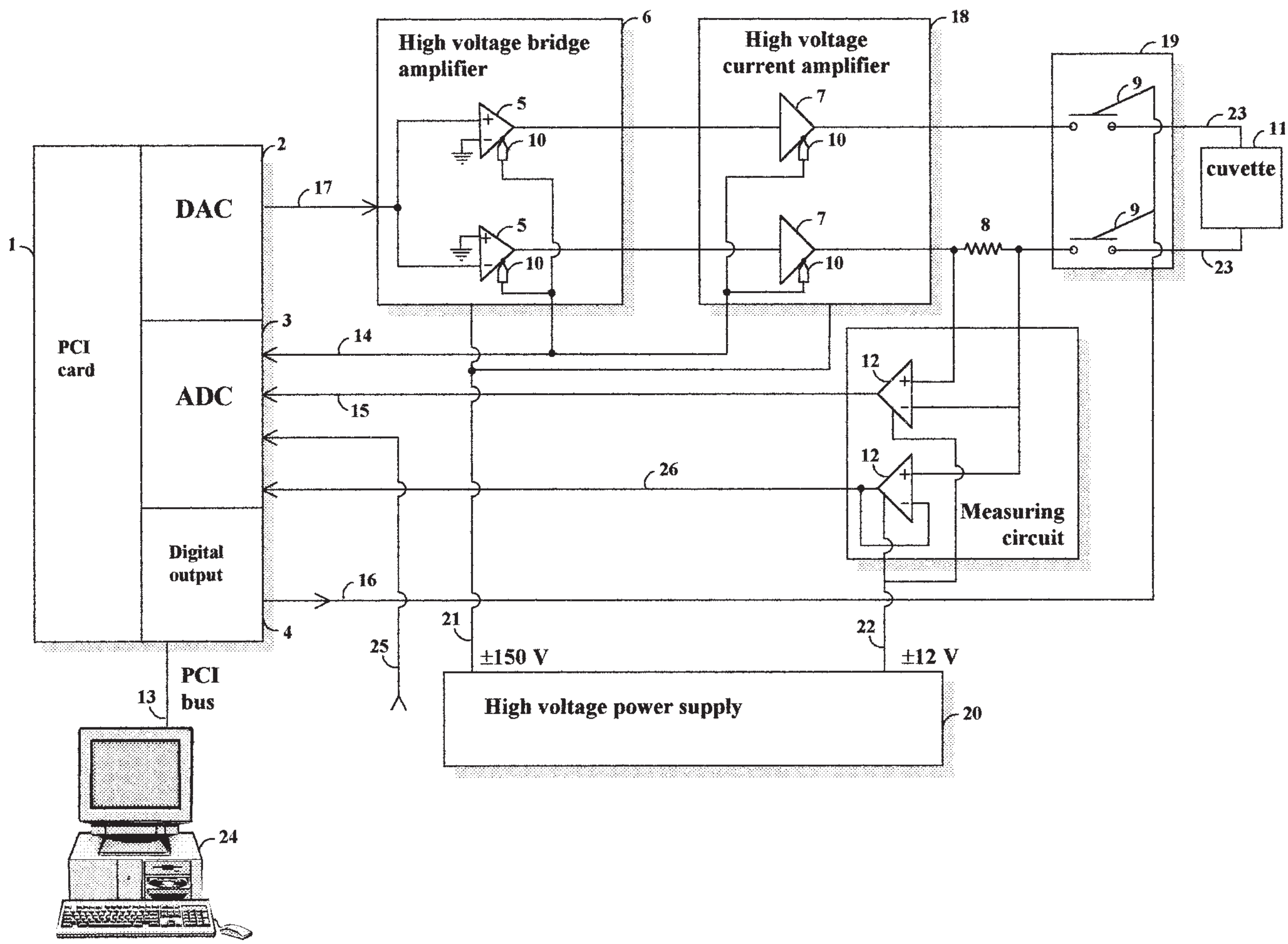


Figure 1. *Block diagram of the DPS electroporator. 1. Analog-to-digital converter (ADC)/digital-to-analog converter (DAC) PCI card. 2. DAC. 3. ADC. 4. Controller with digital output. 5. Microchip. 6. High-voltage, high-speed amplifier. 7. Microchip. 8. Measuring resistor. 9. Switch. 10. Thermosensor. 11. Electroporation cuvette. 12. High-speed, low-voltage amplifier. 13. PCI bus. 14. Input line for temperature measurements. 15. Input line for current measurement. 16. Output control line for relay control. 17. DAC output, forming electroporation pulse shape. 18. High-voltage high-speed current amplifier. 19. Relay. 20. High-voltage power supply. 21. Output of high voltage power supply. 22. Low-voltage output of power supply. 23. Electroporator output. 24. PC computer. 25. Input for monitoring of external porators. 26. Input line for amplitude measurement.*

EP starts with a transmembrane electric field induced by a steeply rising pulse in an external electric field. The latter then causes breakage of cell membranes, thus forming electropores, but only when electric field parameters exceed certain threshold values. In particular, the external field-induced transmembrane potential V should exceed about 1 V[1]. The breakage underlying EP caused by the external pulsed electric field is commonly termed the reversible electric breakdown.[18]

The lifetime of a pore is typically tens of minutes depending on the cell type and ambient temperature,[19–21] the membrane then being highly permeable to molecules and ions including relatively large DNA fragments and proteins. To facilitate introduction of charged molecules into the cell from the outside medium while the pore exists, a low-voltage DC signal is used causing electrophoresis.[22,23] The authors underline that the electrophoresis signal should be generated by the same device as the one used for poration. However, this option is not

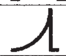
Square (rectangular)	
Line up (increasing)	
Line down (decreasing)	
Sine up (increasing)	
Sine down (decreasing)	
Exponent up (increasing)	
Exponent down (decreasing)	

Figure 2. Basic pulse shapes preprogrammed in the DPS electroporator.

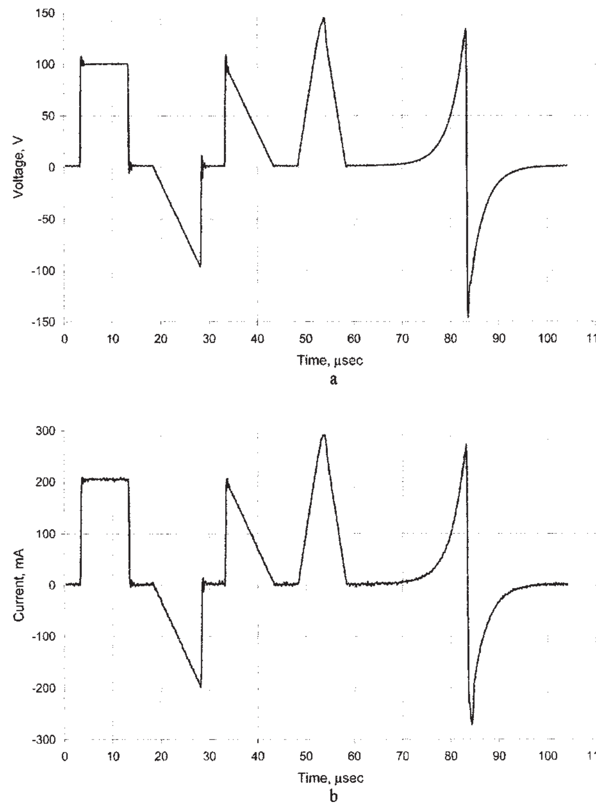


Figure 3. Time plots of voltage (a) and current (b) for passing the pulse group described in Table 2 through the 1000 Ω load.

implemented in the available electroporators[24] because it is an extremely technically difficult task. In the DPS device described in this paper, this option is implemented.

When membranes of two or more cells are in close contact, pore formation caused by EP will fuse two or more cells into one large cell[8] termed a *cybrid*. Indeed, when pores are formed in the membranes of two neighboring cells, those pores can combine into an intercellular through pore, which could further enlarge to reach the size comparable with the pore size, thus forming the cybrid.

Close contact between the cells, or cell bunching, required for successful electrofusion, might be reached by mechanical micromanipulation. However, electrically caused dielectrophoresis and alignment will also result in cell bunching. Cell bunching, together with EP and other related electromechanical phenomena, is generalized in a unifying term, *electrotreatment*.[25–27] The AC field used for cell bunching will be termed *Alignment AC*.

EP can also be used to control the cell cycle: for example, to activate oocytes. That capability is used in mammalian cloning technology.[6,7]

A new field called electrochemotherapy[26,28] was recently developed in medicine. In electrochemotherapy, a chemotherapeutical agent is introduced into target cells through pores induced by EP. In this case, electrodes are inserted into the target tissue or organ. Flow EP devices were developed to treat blood cells. Their functioning is based on repetitive automation control.

Different electroporators were developed by 1997,[24] but electroporators dedicated for a specific task have tended to dominate the market while multipurpose low-cost devices are commercially unavailable. According to the Web site of one of the leading EP device manufacturers, Cytopulse (available at http://www.cytopulse.com/catalog.pdf), such versatile devices are still absent in the EP device market.

All the electroporators cited therein are based on capacitor discharge through the load, which includes the porated cell or tissue. The load through which the capacitor is discharged is biological, so its resistance is difficult to control. This results in the inability of capacitor discharge—based porators to set and maintain pulse parameters with high enough accuracy; yet such accuracy is crucial, particularly when repetitive and/or continuous protocols need to be implemented. Besides, capacitor recharge is a time-dependent process, so the next pulse cannot be applied to the load before a certain time has elapsed since the previous pulse, the delay before the pulse therefore becomes unavoidable, and is typically about 5µs.

Capacitor discharge cannot provide long enough pulses, because the voltage between the capacitor plates

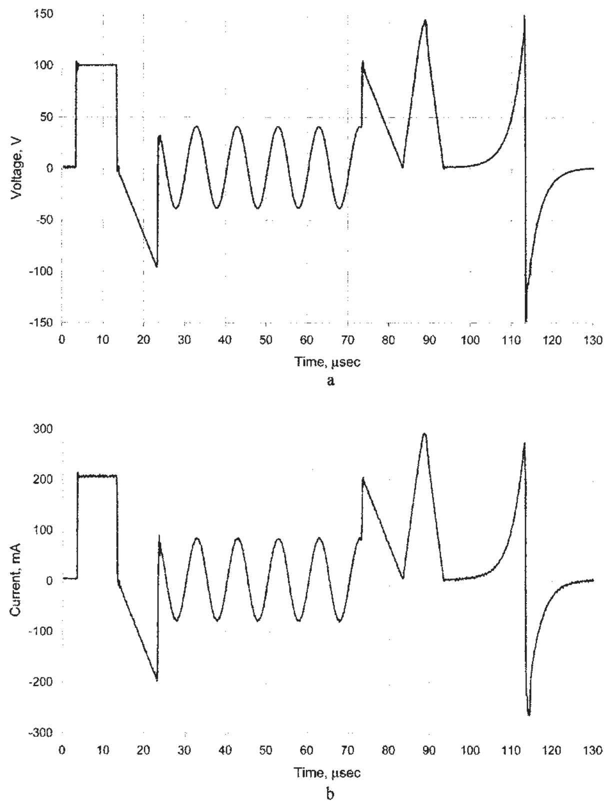


Figure 4. Time plots of voltage (a) and current (b) for the pulse group described in Table 1, with the 40-V 100-kHz sinusoidal AC signal added and delays changed to 3, 0, 50, 0, 0, and 0 µs for the six consecutive pulses, respectively.

will fall during the capacitor discharge. Accordingly, an additional low-voltage pulse generator is required as an add-on to known EP systems if the protocol requires electrophoresis of ions into the cell with the newly formed pores.

In the capacitor discharge-based devices, the capacitor is connected to the load by a high-speed switch, which provides a sufficiently fast slew rate of both rising and falling voltage in the generated pulses. Some of the cited porators provide computer control of that switch by a digital-to-analog converter (DAC) card. However, none of those devices utilizes a DAC card directly for generating EP pulses, so those devices are intrinsically unable to generate arbitrarily programmable pulse shapes. The latter option is provided only in the DPS system described herein.

Only rectangular and exponent decay pulse shapes are provided in available porators. The need for more complex shapes is illustrated by the recent improvement of both EP and electrofusion efficiency by using oscillating electric field instead of a DC field to treat the cells.[29–31] Accordingly, oscillating EP pulses, or AC-modulated DC pulses, have advantages over plain DC fields because the oscillating field-induced transmembrane potential is not dependent on the cell size, and can be used to uniformly porate the intrinsically inhomogeneous biological objects such as liposomes. EP of inhomogeneous cell population, or cells with irregular morphology, by DC pulses is difficult to optimize. In contrast, it is possible to use the same pulse of AC field to porate the cells of different sizes or cells with irregular morphology.

Moreover, if the AC field used to porate the cells is applied simultaneously with the DC field used for bringing cells closer together (during fusion) or introducing charged macromolecules such as DNA into the cell (during transfection), this should be expected to improve the fusion or transfection rate.[32,33]

This brings about the need for electroporators with arbitrary pulse shapes, including AC-modulated bipolar DC shapes. If, additionally, such a device will be fully automated or computer-controlled, this will be particularly useful when repetitive protocols are required, as, for example, in flow EP of blood cells.[34–36] For such tasks, complete computer control of EP is required, so the software will be used to calculate pulse parameters for concrete experimental conditions, as well as to monitor the pulses and to log EP/fusion records and entire protocols to a hard disk. When retrieved, those data will provide the ability to reproduce experiments and run long-term repetitive protocols.

All of these features are present in the new type of electroporator, the Digital Poration System (DPS) that we designed, constructed, developed, and describe in this paper.

System Design and Construction

Figure 1 shows the design of the DPS system as a block diagram, partially simplified for clarity. Because the main distinguishing feature of the DPS system compared to other available electroporating systems is the pulse generator, major functional blocks and interconnections of the pulse generator used in DPS are shown. The EP chamber and electrode design is similar to EP systems described elsewhere.[24]

In terms of major functional blocks, the DPS design consists of the analog-to-digital converter (ADC)/DAC

PCI data acquisition card (1), the high-voltage, high-speed bridge amplifier (6), the high-voltage, high-speed current amplifier (18), the relay (19), and the EP cuvette (11). Two high-speed, low-voltage amplifiers (12) used for monitoring and high-voltage power supply (20) are also shown.

The ADC/DAC PCI card (1) inserted into the PC computer (24) generates a low-voltage pulse with a user-programmed shape. The low-voltage DAC pulse is amplified by high-voltage, high-speed bridge amplifier (6). The high-voltage pulse from the bridge amplifier output is amplified by current using the high-speed, high-voltage current amplifier (18). The low-impedance resistor (8) provides the signals for current and amplitude monitoring of output pulses. Those signals are amplified by low,voltage, high-speed amplifiers (12), and then digitized by the ADC card. The relay (19) with switches (9) disconnects the EP chamber (11) from the amplifier output to prevent possible sample damage by residual low-voltage output—shifted "zero" voltage of the amplifier (18). The relay (19) is controlled by the digital output (4) embedded in the PCI ADC/DAC card (1). The power supply 20 supplies high voltage for the high-voltage amplifiers as well as low voltage for the monitoring amplifiers. The temperature of the microchips is monitored by thermosensors (10). The signals of the thermosensors (10) are digitized by ADC (3) and controlled by the DPS software.

EP pulse parameters are preset by the software. The main distinguishing feature of this electroporator is that a computer-driven DAC card is used as the primary pulse generator, with the pulse to be further amplified by both voltage and current. This allows the application of arbitrarily shaped pulses to the load. Moreover, those pulses are fully programmable in a point-by-point manner via the appropriate user interface of the software pertaining to the device. Other tasks of the computer include monitoring and logging of the currently running EP protocol. Again, the fashion in which the ADC/DAC card is used in this work allows the protocols to be logged with very high precision in the digital form, and then retrieved to be exactly reproduced in further experiments. Thus, two purposes are served: general reproducibility of the results, and ability to program long-term repetitive protocols such as those used for electrochemotherapy purposes.

To achieve high speed of the amplifiers, a specially designed and constructed four-layer printed board is used. The safety systems include overheating protection for microchips, current fusing, detection of circuit breakage (especially for contacts with the load) and output short circuit. The relay connected to the amplifier output ensures that high-current pulses are on during short periods only, to meet the safety requirements for the device operator and possibly for the patient.

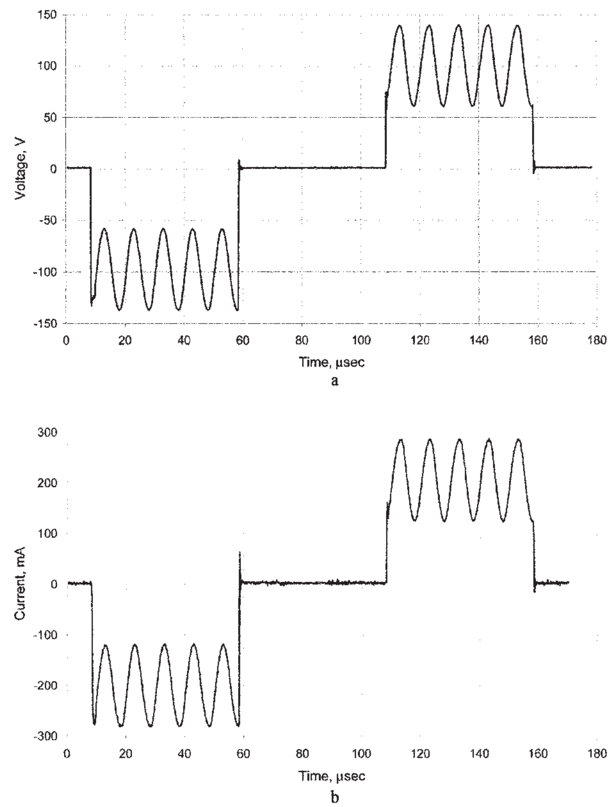


Figure 5. Time plots of voltage (a) and current (b) for two consecutive 100-Volt 50-μs rectangular DC pulses passed through the load with a 50-μs pause between them. Both pulses were modulated by 40-V, 100-kHz sinusoidal alignment AC.

The user interface of DPS allows programming of a group of up to 6 pulses. For each individual pulse in the group, the user can set the shape, amplitude, duration, polarity, and time delay before the pulse. The shape of each pulse in the group can be selected from the shapes shown in Figure 2. Those shapes include line, sine, and exponent, all of which can be set to be either increasing or decreasing, as well as rectangle and triangle. Alternatively, the shapes can be loaded from a user-created text file with a simple structure. In the latter case, the program interpolates the voltage of field strength values between the points specified in the user file, allowing to fully utilize the performance of the high-speed DAC card.

The program also allows the user to superimpose an AC signal onto the prepared pulse group, as well as to selectively modulate each pulse in the group, or any of the pauses between pulses in the group, by AC signal. Frequency, shape, amplitude, and duration values of the AC signal are set by the user. All shapes available for EP pulse shape selection are available for AC signal shape selection as well. Besides, groups of EP pulses can be accompanied by the relatively long (seconds to minutes) bipolar AC signal used for aligning the cells, with user-defined pulse shapes, amplitudes and frequencies, to be applied before and/or after the group of AC pulses.

The output signal can be run either by pressing a hot key or by depressing a pedal. Repetitive generation of pulse groups according to a predefined protocol, as well as protocol saving to and loading from a disk file, are also implemented.

DPS can generate batches of EP pulse groups possibly enclosed by alignment AC signals, with the parameters of both EP pulse and alignment AC set in the previous modes. The batches can be separated by long (fractions of minutes to many hours) programmable time intervals.

Performance

Performance characteristics of the most important functional blocks of DPS are shown in Table 1. Those characteristics allow the user to implement an EP device able to generate high-voltage (>100 V) pulses with arbitrarily programmable shapes, with sufficiently high slew rates (about 1 V/ns) and sufficiently high currents (>1 A) for EP purposes.

In Figures 3–5, performance of the DPS system on a 1000 Ω load is shown. In the example of Figure 3, the system generates a group of six consecutive DC pulses. For each of the pulses, parameters are set independently and listed in Table 2. The DPS system is used for both pulse generation and monitoring, including voltage, current, and temperature (the latter is not shown). Figure 3a shows the voltage, and Figure 3b the current flowing through the load.

In the example in Figure 4, six consecutive pulses are programmed as in the example in Figure 3. Their parameters remain the same as for the previous example (Table 2), with the exception of the delay values. In this example, delays of pulses 2, 4, and 5 were set to zero, while the delay for pulse 3 was set to 50 μs. Also, an additional alignment AC signal is added to the DC pulses, in the "Between pulses" mode. In contrast, no AC signal is added in the previous example. In the example in Figure 4, the AC frequency is 100 kHz, the amplitude is 40 V, and the AC period shape is sinusoidal. The latter shape is selected for simplicity only. In fact, the DPS system can generate the same choice of AC period shapes as the choice of shapes available for generating DC pulses. Figure 4a shows the voltage and Figure 4b the current.

The example in Figure 5 contains two consecutive 100-V, 50-μs rectangular DC pulses with a 50-μs pause between them, with alignment AC in the "During pulses" mode; that is, the DC pulses are modulated by the AC alignment signal. As in the previous example, a 40-V, 100-kHz sinusoidal AC signal is applied.

The limiting values of pulse and pause duration are determined by the performance of the particular model of DAC/ADC card incorporated into the DPS device. Typical performance parameters are 10 to 30 MHz and 16 Kbytes, which allows generation of pulse groups 5 μs to 0.5 ms long, with 0.03 μs resolution. Longer pulses of up to several seconds are possible, but with poorer resolution.

DeFrancesco[24] cites operation parameters of several commercially available electroporators. In Table 3, these parameters of DPS are compared with those of electroporators supplied by the two most advanced manufacturers, BTX and CytoPulse. As follows from the abovementioned report by CytoPulse, that comparison remains valid as late as 2003.

From Table 3, the following features distinguishing DPS from the commercially available electroporators are easily seen. First and foremost, DPS is the only electroporator with arbitrary, user-programmable pulse shape, thus having the obvious advantage in flexibility.

The pulse length range is maximal for DPS in terms of orders of magnitude. Indeed, it is 6 orders of magnitude against the maximum of about 4 for the BTX ECM-600 model, and about 3 for most other porators. With respect to the minimal available pulse length, DPS compares favorably with other porators (0.1 μs versus the minimum of 0.3 μs).

The unmatched zero in the Charge Time column is due to the fact that the DPS is not capacitor discharge-based. The actual meaning of that parameter is the minimal, unavoidable delay between pulses, because no new pulse may be started until the capacitor is recharged. In DPS, this drawback is completely removed.

A disadvantage of DPS is also evident from Table 3, which is the voltage span, 500 V for DPS compared to about 3,000 V for the BTX models. Therefore, the limitations of DPS application are both the maximal voltage and the minimal pulse length; these prevent DPS from being used for generating the very high-voltage nanosecond pulses required for poration of membranes of smaller intracellular organelles.[37–40] The voltage limitation can be relieved by decreasing the distance between the electrodes in the EP chamber, as well as by decreasing the electrode size. This means that the same protocols as those realized using the 3,000 V BTX device can also be realized using DPS, but with smaller volumes of the medium between the EP chamber electrodes.

Some other parameters not included in DeFrancesco's[24] analysis are crucial for EP efficiency. These include the slew rate and the precision of setting and measuring EP parameters. As specified above, the most important operation parameter of the electroporator is the slew rate, which is the electric field increase/decrease rate. Indeed, if the slew rate is not fast enough to prevent discharge of the biological membrane while it is being charged by the poration pulse, EP is unlikely to occur at all. The typical value for most mammalian somatic cells is about 1 V/ns. In DPS, this slew rate is achieved with the electrical characteristics of DAC and the amplifiers listed in Table 4. The 500-V limitation in the voltage range is in fact determined by the ability of the available electronic components to charge low loads (100–500 Ω) to such high voltages with sufficient slew rates).

In DPS, precision values for setting and measurement pulse parameters are 0.1 V with the voltage set at 250 V, and 0.1 μs step size for pulse duration and delay using DAC with 14 bit resolution and 30 MHz sampling rate.

Discussion

In this work, we designed, constructed, and tested DPS, a digital EP system in which the pulse for EP is formed by DAC in a point-by-point manner. This is the main distinguishing feature of DPS in comparison with known electroporators in which the pulse is generated by means of capacitor discharge. The predecessors of this novel feature come from two fields. On the one hand, the point-by-point signal formation of DAC is well known and widely used in electronics. This method, however, was never used to create pulses to be delivered to a biological

Table 1. Performance data of the main electronic elements of DPS.

High-Voltage, High-Speed DC Amplifier
Output DC voltage: ±250 V
Gain: 200
Input voltage: ±2V
Slew rate: 1 V/ns or better
Load: 1 kΩ
Current protected
DC power: ±150V at 0.7 AOutput accuracy: 0.2%
Input resistance: 50 Ω
Low-Voltage Differential Linear Pulse DC Amplifier
Maximum output DC voltage: ± 2.5 V
Frequency: DC-30 MHz
Gain: 1–50 programmable by resistors
Slew rate: 50 V/ns or better
Load: ≥10 kΩ
Input current: <5 μA
Input resistance: ~30 kΩ
Output resistance: 10 Ω
DC power: ±12 V
Stabilized power supply
Line insulated outputs:
1: +12 V at 0.5 A over current, self-protected
2: –12 V at 0.5 A over current, self-protected
3: + 150 V at 5 A over current, self-protected
4: –150 V at 5 A over current, self-protected
5: ground
Line fuse
Input voltage: 110/220 VAC 50/60 Hz
Ripple: 1 V P/P noise
Analog-to-Digital Converter
Resolution: 14 bit
No. of channels: 16
Sampling rate: better than 10 MHz
Full scale input: ± 2.5 V
Input impedance: 1 MΩ
Input capacitance: 60 pF
Maximum input voltage: 2.5 V
Digital-to-Analog Converter
Resolution: 14 bit
No. of channels: 1
Sampling rate: 30 MHz
Full-scale output: ± 2.5 V
Output impedance: 50 Ω
Output voltage settling time: 40 ns
FIFO memory: 128 K

load for EP purposes, because only in recent years have ADC/DAC cards with sampling rates and output voltage settling times sufficient for EP become available.

On the other hand, there have been attempts to use ADC/DAC converters to control the protocol of EP.[41–44] In these patents, DAC is used to control the generator of the EP pulse in various manners. For example, an electroporator device is described[42] in which ADC/DAC is used to control the capacitor switch, thus determining duration of capacitor discharge on the load and duration of pauses between pulses.

However, ADC/DAC cards have never before been used to create the exact shapes of poration pulses by a point-by-point method, with those pulses then delivered to the load. In DPS, DAC does not *control* the pulse shape generator but instead *is* that signal generator. It is the DAC-generated signal that, after being amplified by amplitude and current while its shape is being repeated, is delivered to the load. The DPS uses the DAC to generate pulses with arbitrary programmed shapes for EP purposes, as well as those of related phenomena such as cell fusion, dielectrophoresis, electrorotation, or separation. Below, we shall describe the implications of the novel EP features implemented in DPS.

Arbitrarily Programmed Pulse Shapes

EP treatment by rectangular or exponent decay-shaped pulses is known to be commonly accompanied by high rate of cell lethality.[45,46] In this connection, it is desirable to use special pulse shapes different from rectangular and exponent decay shapes in order to avoid cell damage, thus increasing EP efficiency. However, as already mentioned, a device based on a capacitor discharge can not generate an arbitrarily programmed (unrestricted) pulse shape, which limits the choice of parameters and hampers optimizing pulse shapes. To the best of our knowledge, while some porators have certain pulse parameter flexibility—that is, they are able to program pulse amplitudes, lengths, and intervals between the pulses—they can neither vary the actual shapes of the pulses nor modulate them with AC signals used for cell alignment.[24] Such flexibility is implemented in the DPS porator.

Bipolar Pulses

Unlike most other porators,[24] DPS provides bipolar pulses. The only other electroporator known to us that provides such pulses is described by Puc et al,[47] who point out the importance of such pulse. Indeed, for only a bipolar pulse, its integral over time may equal zero. Such a pulse will transfer zero charge, and will not alter ionic content inside the cell when EP for cell fusion is complete. Another application of bipolar shapes is cell fusion, in which it is vitally important to preserve the ionic contents of the cell, which strongly increases viability of the cybrid obtained after fusion. As shown by Rols and Teissie,[48] increasing the ionic strength of the pulsing medium results in an increase in sieving of transient permeant structures, but decreases the fusion rate. Besides, the positive and negative parts of EP pulses can be made either identical or different in DPS, which may be important for several types of EP procedures. During EP used for delivery of various charged agents (such as DNA in transfection) into the cell, this is achieved by applying a DC pulse with sufficient amplitude to ensure electrophoresis through the formed pores. Those agents may have different charge signs. Then, again, bipolarity of the pulse becomes crucial. Moreover, shapes of positive and negative parts of the bipolar pulse may need to be different because electrophoretic properties of the molecules to be introduced into the cell are different. The latter properties are affected by molecule sizes, charge values, dipole moments, translational and rotational diffusion coefficients, etc. Thus, subtle effects of chemical agent delivery into the cell may be controlled and programmed by varying the positive and negative parts of a bipolar pulse.

Table 2. Parameters of the pulse group for which time courses of voltage and current are shown in Figure 3.

No.	Delay, m s	Length, m s	Amplitude, V	Pulse Shape
1	3	10	100	Rectangular
2	5	10	–100	Linear increasing
3	5	10	100	Linear decreasing
4	5	10	150	Triangular
5	5	20	200	Exponential increasing
6	0	20	–200	Exponential decreasing

Alignment AC

The DPS system enables application of high frequency AC to cells before, during, between and after EP pulses. For example, AC can be used for prealigning asymmetrical cells into a similar orientation, so that all cells will be subjected to the same transmembrane EP pulse. This will ensure uniformity of pore formation among the cells. According to the EP theory described by Whelan,[9] uniform pore formation leads to smaller cell lysis rates.

Application Prospects

The DPS system implemented in this work is, in effect, a generator of six-pulse groups containing bipolar pulses of arbitrary shapes, programmed independently for each pulse in the group. Unlike other porators, in DPS lengths of pauses between pulses in each group are arbitrary, because capacitor discharge is not used to generate pulse shapes. Varying the pause length between two pulses of different polarities, with steep front and back edges respectively, will allow researchers to investigate relaxation prospects in the membrane or entire cell with the corresponding relaxation times. Particularly, the microsecond time range typical for dielectric relaxation of the membrane[49] becomes accessible for detailed investigations.

Also, periodic signals (batches) can be generated, with the period being the six-pulse group mentioned. Besides, DPS is able to generate an additional AC signal used for the so-called cell alignment before, after, or between any pulses in the group, as well as simultaneously with those pulses, thus modulating them.

Due to its inherent ability to vary the shapes of the generated pulses, the DPS system allows the user to optimize the shapes of those pulses for particular EP tasks. In particular, DPS allows the generation pulses with slowly rising voltages and subsequent steeply falling edges, the slew rate of the edge being about 1 V/ns. One example is the “line increasing” shape in Figure 2. For such pulse shapes, pore formation is not expected to occur during application of the linearly rising part of the pulse. EP application of such pulse shapes has not previously been described in the literature. The other unique pulse shape provided by DPS, the “line decreasing” shape (Figure 2), does not have the steep fall at the back edge of the pulse. Investigation of such pulse shapes should improve first the understating of EP mechanisms, and second, EP efficiency.

Bipolar pulse shapes, compared with corresponding unipolar shapes of the same amplitude, should lead to the same poration rates.[50] On the other hand, bipolar

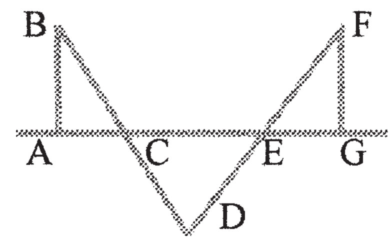


Figure 6. Pulse shape differing from rectangular shape by bipolar signal between the steep rise and the steep fall.

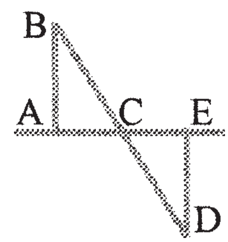


Figure 7. Bipolar pulse shape with two steep rises and no steep falls.

shapes, if the time integrals of their parts with different polarities are equal in absolute values, should transfer no charge across the cell membrane, thus rendering no change to the ionic content of the cell and increasing the cell viability.

In Figures 6–7, pulse shapes expected to separate the contribution into EP of steep voltage rises and falls are shown. The pulse shape shown in Figure 6 should be more efficient compared to a rectangular pulse. In this pulse shape, the bipolar shape shown as BCDEF is introduced between the AB and FG parts instead of the constant voltage DC signal as in the rectangular pulse. The area of triangle CDE equals the sum of the areas of triangles ABC and EFG. Thus, only the the AB and FG parts will porate the membrane, while the overall charge transferred across the cell membrane will be zero.

Figure 7 shows the pulse shape differing from the rectangular shape in two aspects. First, as in the previous shape, a bipolar (BCD) signal is introduced instead of the constant-voltage DC signals, and second, both steep edges are rises, in contrast to the rectangular shape in which those edges have opposite directions. Such pulses reveal the role of oppositely-directed steep edges in EP pulses in EP and related processes, including their influence on the induced transmembrane potential.

Table 3. Comparison of operation parameters for commercially available electroporators with those of DPS.*

Manufacturer and Model	Pulse Shapes Available	Voltage Range, V	Pulse Length	Charge Time, s	Biological Application	Special Features
BTX T-720	Exponential	50–2,500	5–6 ms	<30	Bacteria	
BTX ECM 395	Exponential	50–3,000	5–135 ms	<25	Bacteria, animal	Personal Electro Pak
BTX ECM 600	Exponential	50–2,500	0.3 ms–2 s	<5	All cell types	Larger pulse lengths
BTX T-820	Rectangular	50–3,000	0.3 µs–99 µs	<5	Animal, plant	Rectangular wave
BTX ECM 2001	AC and rectangular	10–3,000	0.3 µs–99 µs	<5	Fusion/poration	Combined functions
CytoPulse PA-2000	Rectangular	50–1,100	1 µs–1,000 µs	6	Bacteria, animal, plant	High field cuvette
CytoPulse PA-4000S PulseAgile	Rectangular	50–1,100	1 µs–1,000 µs	6	Bacteria, animal, plant; suspension or tissue	PulseAgile
CytoPulse PA-4000F	AC and rectangular	50–1,100	1 µs–1,000 µs	6	Bacteria, animal, plant; suspension or tissue; cell fusion	PulseAgile
DPS	7 basic pulse shapes: rectangular, linear down, linear up, exponential down, exponential up, sinusoidal down, sinusoidal up; also, their combination, their superposition, and arbitrary, user-programmable shapes	±3–±250†	0.1µs‡–100 ms	0 (always ready for new pulse)	All cell types; ideal instrument for cell alignment, CSR, repetitive protocols, fusion, mimicking calcium waves, electrophoresis, eletrochemotherapy, cell separation	7 basic pulse shapes, plus their combinations and superpositions; arbitrary, user-programmable shapes; alignment AC before, between, during, and after EP pulses; low voltage signal for electrophoresis; groups of up to 6 pulses; batches of groups

*Data are partly quoted from DeFrancesco.[24]
†The pulses may be bipolar. The resulting voltage span is 500 V.
‡When this value is used, two types of limitations may be encountered: (1) of DAC sampling rate, and (2) of disk space if long protocols are recorded. Both follow from point-by-point formation and high-speed digitizing of the signal.

The DPS system was initially designed and constructed to be dedicated for use in mammalian cloning technologies, for the specific procedures of somatic cell electrofusion with an enucleated oocyte and mimicking calcium waves during oocyte activation. The actual system turned out to be an exceptionally versatile electrotreatment device capable of performing many modes of electric field action on biological objects, and quite promising for a wide variety of applications, including all the above-listed electrotreatment effects. The DPS system is equally convenient not only in both EP-related fields such as electrofusion, transfection, and electrochemotherapy, but also in a wider selection of research and technology fields that can be generally termed *electromagnetobiology* and includes dielectrophoresis of cells, electrophoresis of cells and tissues, cellular "spin resonance" (electrorotation), cell manipulation, sorting, and selection.

The application range of the developed DPS system is also considerably widened due to presence of a multichannel ADC card in the design. For instance, EP protocols may easily be optimized by measuring the current through the newly formed pores once the EP pulses have been applied. In this context, a combination of DPS with patch clamp techniques should be quite informative for investigating EP-induced cell membrane conductivity as done by Ryttsen et al.[51]

The prospects of DPS applications in medicine deserve special attention, because some features of DPS, particularly its ability to generate low-voltage DC signals during long times after the EP pulses and its ability to run multiple repeated protocols for EP of the bloodstream,[34–36] make it very promising in electrochemotherapy and gene therapy. Generally, procedures of tissue stimulation should benefit from pulse generation procedures that avoid capacitor discharge, because the pulse action on the tissue will then be less dependent on tissue resistance.

In addition, if many electrodes are used for EP, the digital output available on the ADC/DAC card makes it possible to easily control switchboards that distribute electric field pulses over electrodes, which may be needles or rods arranged in a 3D array. The latter electrode configuration is now increasingly used in tissue and organ electrochemotherapy in vivo,[52–54] so DPS may be used to optimize drug and gene delivery protocols for such configurations.

Table 4. Performance characteristics of DAC and the high-voltage, high-speed current amplifier ensuring the 1 V/ns slew rate in DPS.

Parameter	Value
DAC	
Sampling rate, MHz	30
Settling time for DAC output voltage, ns	40
Resolution, bit, better than	12
High-voltage, high-speed current amplifier	
Slew rate,V/ns	1
Output frequency band, MHz	DC to 6.25
Maximal output voltage, V, better than	±250

Conclusions

We have developed DPS, an EP system in which EP pulses are initially generated by a DAC. This has a number of advantages, each of them having biological significance. First, EP pulse shapes in DPS are not restricted to rectangular and exponent decay pulse shapes, as has been the case in all EP systems commercially available to date. Instead, arbitrarily programmable shapes that can be entered on a point-by-point basis are implemented.

The new pulse shapes implemented in DPS also allow the EP protocols to be optimized for a wide range of EP applications in a single instrument, including electrofusion, transfection, tissue stimulation, and oocyte activation by mimicking calcium waves.

In addition, bipolar pulses are implemented that increase viability of the porated cells. We found that efficiencies for bipolar pulse shapes are higher than for the corresponding unipolar shapes.

Compared with all porators commercially available at present, the operation parameters of DPS are equivalent or superior, with the sole exception of voltage span, which is 500 V, compared to about 3,000 V for some available models.

The use of high-speed, high-voltage bridge amplifiers together with high-speed, high-voltage current amplifiers in EP devices enables the EP pulses generated by DAC to be amplified to sufficient voltages and currents with a slew rate of about 1 V/ns, which is crucial for EP of most cell types.

There is no requirement in DPS for any external device such as the commercially-supplied "optimizers"[55,56] which are used for monitoring pulses because in DPS, ADCs are utilized to monitor EP pulses. Thus, the

DPS electroporator is completely digital and computer-controlled, which enables automation of single as well as repeated EP processes and allows the user to program and log very long repetitive protocols.

The EP pulses may be modulated by or combined with an alignment AC signal generated by the same DAC card included in DPS, to orient and to bring and press cells together before, during, or after pore formation. One application of these effects is cell fusion.

The software for the computer-based EP system provides a user-friendly interface, allowing the user to record protocols for EP and other types of electrotreatment and recalculate all parameters of pulses for concrete EP conditions when those protocols are retrieved.

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