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Review Article

Recent advances in *in vivo* electroporation technologies for therapeutic and biomedical applications

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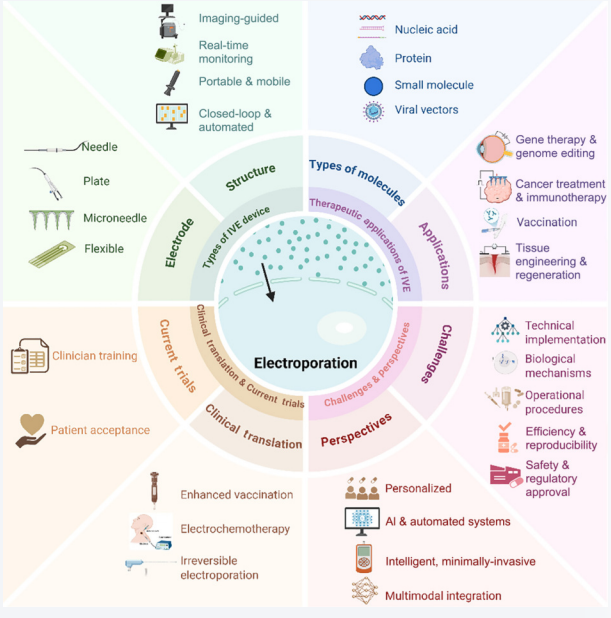
ABSTRACT: *In vivo* electroporation (IVE) has gained significant attention as an efficient, versatile, and minimally invasive technique for intracellular delivery of nucleic acids, proteins, and other biologically active molecules. By applying electrical pulses to transiently permeabilize cell membranes, this technique enhances the uptake and expression of therapeutic agents, enabling diverse applications in gene therapy, cancer immunotherapy, vaccination, tissue regeneration, and genome editing. This review summarizes recent advances in electroporation technology, including innovations in device design, optimization of pulse parameters, and combinatorial strategies, with a focus on translational biomedical research. Key challenges such as tissue response heterogeneity, safety concerns, and reproducibility are also discussed. By examining ongoing clinical trials and future directions, we aim to offer insights into addressing these barriers and accelerating the clinical translation of *in vivo* electroporation for therapeutic use.



KEYWORDS: *in vivo* electroporation, non-viral delivery, cancer immunotherapy, vaccination, genome editing, electrode design, clinical translation

1 Introduction

In vivo electroporation (IVE) has emerged as a powerful and versatile technique for delivering biomolecules directly into living tissues. It offers a non-viral, transient, and controllable method for intracellular delivery by applying brief, high-voltage electric pulses to transiently permeabilize cell membranes [1, 2]. This allows macromolecules such as plasmid DNA, RNA, proteins, and small-molecule drugs to cross the lipid bilayer and enter target cells efficiently [3]. Initially developed as a research tool to enhance gene transfer in animal models, IVE has matured into a clinically relevant platform with applications spanning gene therapy, cancer immunotherapy, DNA and RNA vaccination, regenerative medicine, and genome editing [4].

Conventional delivery platforms are primarily chemical or biological in nature, often relying on lipids, polymers, or viral vectors to carry and protect therapeutic cargo. While these systems offer advantages such as cellular targeting and high packaging efficiency, they are often limited by toxicity, immunogenicity, complex manufacturing, and lack of spatiotemporal control [5]. Electroporation, by contrast, is a physical method of delivery that operates independently of receptor-mediated uptake or endosomal trafficking. This makes it particularly attractive for localized, on-demand administration of therapeutics-an area where chemical and

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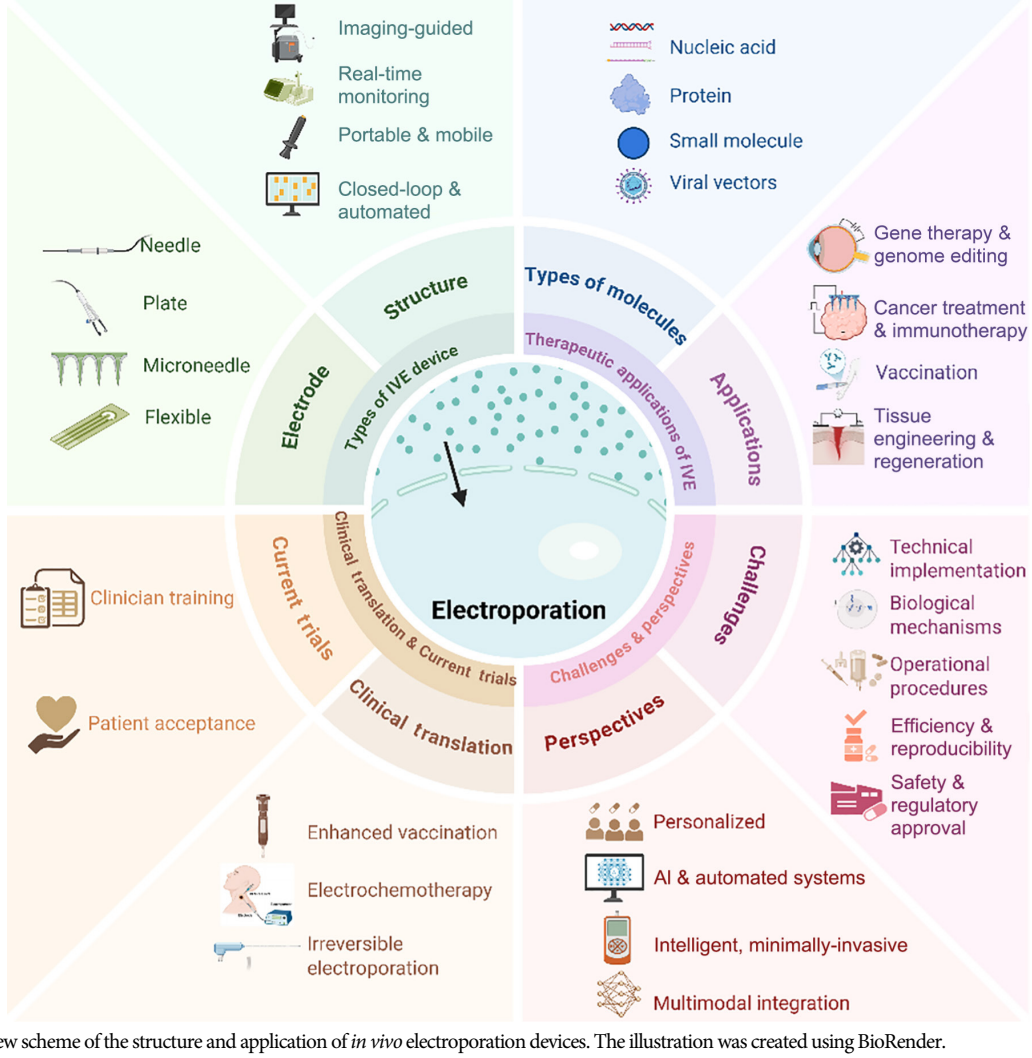
biological systems frequently fall short [6, 7]. IVE therefore fills a critical gap in the delivery strategies, providing an alternative modality for cases where conventional systems are ineffective or impractical.

Recent technological advances have substantially expanded the capabilities of IVE. Innovations in electrode design-including microneedle arrays [8], stretchable substrates [9], and imaging-guided applicators [10] have improved the precision, safety, and patient tolerability of the technique. Simultaneously, refined pulse modulation strategies allow for tunable permeabilization profiles tailored to different tissue types and molecular cargos, to ensure effective and uniform molecular delivery [11, 12]. As a result, the range of deliverable agents has broadened significantly to include not only nucleic acids, but also proteins, peptides, and emerging therapeutics such as circular RNA and clustered regularly interspaced short palindromic repeats and CRISPR-associated proteins (CRISPR-Cas) components [13].

The clinical translation of IVE is advancing steadily, with electrochemotherapy (ECT) and DNA vaccine delivery already validated in human trials [10, 14]. Additionally, electroporation-enhanced immunotherapy and genome editing have demonstrated promising results across various disease models (Scheme 1). For instance, in melanoma, IVE-mediated delivery of CRISPR tools into lymph node T cells significantly enhanced antitumor immunity [15]. In pediatric sarcomas, electroporated chimeric antigen receptor-mRNA (CAR-mRNA) into natural killer (NK) cells improved cytotoxicity without the need for viral vectors [16]. For glioblastoma, electroporation-enabled editing of immune checkpoints in T cells improved their tumor infiltration and persistence [17]. These strategies offer precise, non-viral delivery with improved safety and efficacy profiles in preclinical models [18]. With ongoing improvements in commercial devices and regulatory frameworks, IVE is expected to become increasingly integrated into the clinical landscape. Nevertheless, several challenges remain, including variability in tissue responses, limitations in reproducibility, and the need for intelligent feedback systems to guide real-time treatment decisions.

This review provides a comprehensive overview of the current state and future prospects of IVE. We begin with outlining the fundamental mechanisms and technological foundations of electroporation. Next, we discuss its major biomedical applications, emphasizing clinical progress and emerging therapeutic strategies. Finally, we address unresolved challenges and highlight next-generation innovations — such as artificial intelligence (AI)-driven optimization, wearable devices, and integrated combination platforms — that are poised to advance IVE into a core modality for therapeutic delivery.



Scheme 1 Overview scheme of the structure and application of *in vivo* electroporation devices. The illustration was created using BioRender.

2 Fundamental principles and mechanisms of IVE

IVE relies on the application of brief, high-intensity electrical pulses to transiently permeabilize cellular membranes, facilitating direct intracellular delivery of therapeutic molecules [1, 19]. At the core of electroporation is electric-induced membrane disruption, governed by interactions between applied electric fields and the biological lipid bilayer [1, 2]. When the transmembrane voltage ΔV_m ~ 0.2–1.0 V [20], transient nanoscale pores begin to appear in the lipid bilayer, but membrane integrity is restored within seconds to minutes. When ΔV_m exceeds 1 V, the membrane pores continue to expand, leading to ion leakage and cell death [18, 21]. However, the magnitude of the induced ΔV_m is also dependent on cellular properties including size, shape, membrane composition, and orientation relative to the applied electric field [18, 22]. Upon exceeding this threshold, transient nanopores form, significantly enhancing the permeability of the cell membrane [23–25] and enabling molecules such as nucleic acids, proteins, and small molecules to enter cells [26–28].

2.1 Molecular mechanisms of membrane permeabilization

At the molecular scale, electroporation-induced pore formation is a complex biophysical process driven by electromechanical forces that cause rearrangements in membrane phospholipids [29]. Electric pulses induce rapid and localized reorientation of lipid molecules, transitioning transiently from a tightly packed hydrophobic arrangement to create hydrophilic pathways through the membrane. These transient nanopores typically span sizes from nanometers to tens of nanometers and remain open for microseconds to milliseconds, depending on pulse duration and intensity [18]. The dynamics and resealing of these pores are strongly influenced by several membrane properties. Lipid composition affects bilayer integrity and susceptibility to disruption, where unsaturated lipids tend to increase membrane fluidity and pore formation [30]. Cholesterol content stabilizes the bilayer by reducing fluidity, thus increasing resistance to electroporation and slowing resealing [31]. Membranes richer in unsaturated lipids or cholesterol exhibit distinct electroporation thresholds compared to those rich in saturated lipids [32, 33]. Meanwhile, membrane fluidity itself governs the rate and extent of pore expansion and closure [34]. The presence of membrane proteins and underlying cytoskeletal elements, such as actin filaments, contributes mechanical stability, altering pore shape and limiting resealing efficiency [35].

These molecular insights help optimize electroporation parameters to enhance molecule delivery efficiency while minimizing cellular damage and apoptosis.

2.2 Physiological and histological analyses

Unlike isolated cells, electroporation *in vivo* introduces additional layers of complexity due to the physiological environment. Factors such as blood flow, extracellular matrix (ECM) composition, and immune system responses substantially affect electroporation outcomes [36, 37]. For example, blood and blood vessels have higher conductivity than surrounding liver tissue. Therefore, during electroporation, the distribution of the local electric field will be changed, forming an "electric field sink" effect, which reduces the electric field strength in certain areas and thus affects the electroporation efficiency [38, 39]. Additionally, ECM components such as collagen fibers, elastin, and glycosaminoglycans physically impede uniform electric field distribution and molecular diffusion, leading to variability in electroporation efficiency across different tissues and anatomical sites [40, 41]. These physiological barriers not only influence macroscopic treatment performance but also shape microscopic transport pathways and electroporation zones. The skin's stratum corneum provides a classic example of such a physiological barrier. Electroporation can induce transcellular transport pathways within the dense stratum corneum, furthermore, tissue sections provide visual evidence of this auxiliary delivery effect [19].

Tissue-specific properties, particularly electrical conductivity, anisotropy, and cellular density, significantly influence the predictability and effectiveness of IVE. For instance, highly conductive tissues such as muscle and tumors, due to their elevated electrolyte content, facilitate smoother and more uniform current propagation, thereby generating predictable and consistent electric fields for electroporation [42]. In contrast, tissues with low conductivity, including adipose and fibrous tissue, exhibit high electrical resistance, which disrupts current distribution and results in heterogeneous electric fields, ultimately compromising the stability and efficacy of molecular delivery [43]. Anisotropy is another influential factor, especially in structurally aligned tissues like skeletal muscle. Here, current conduction is significantly more efficient along the direction of muscle fibers than across them. As a result, applying the electric field parallel to the fiber orientation achieves effective membrane poration at a much lower voltage threshold, whereas perpendicular application demands substantially higher voltages to elicit comparable effects [44]. These tissue-specific differences underscore the importance of individualized protocol design.

To ensure uniform therapeutic outcomes, it is essential to carefully consider pulse parameters in light of these biophysical properties [44]. For example, pulse duration — ranging from microseconds to milliseconds — directly influences electroporation dynamics, including pore formation, molecular transport, and cell viability [29]. A simulation illustrating how reversible electroporation, irreversible electroporation (IRE), and thermal injury correlate with electric field strength and pulse duration is presented [19]. Shorter, nanosecond pulses may selectively target intracellular organelles or promote apoptosis, while multiple brief pulses can be more effective for gene transfection than a single prolonged pulse [45]. Notably, the optimal pulse duration depends on the size of the delivered molecule and the specific characteristics of the target tissue or cell type. Under a fixed pulse duration (1 ms), the electroporation behavior of cells (non-electroporated, reversible or irreversible) is closely related to the electric field strength. There is no obvious electroporation at low electric field strengths less than 250 V/cm; at moderate electric field strengths (250–1750 V/cm), the proportion of reversible electroporation first increases and then decreases, and the proportion of irreversible electroporation rises at the upper end of this range, indicating that the electric field strength can effectively regulate cell electroporation [19]. This ability to precisely control the extent of electroporation based on pulse parameters and field strength allows for the development of distinct clinical applications, which are broadly categorized into reversible and irreversible techniques. Reversible techniques include ECT as an adjuvant to therapeutics and electro gene transfer for gene manipulation to treat a variety of conditions. Irreversible techniques include tissue ablation as a monotherapy for targeting malignant tissues and cardiac ablation for treating arrhythmias (Fig. 1) [46].

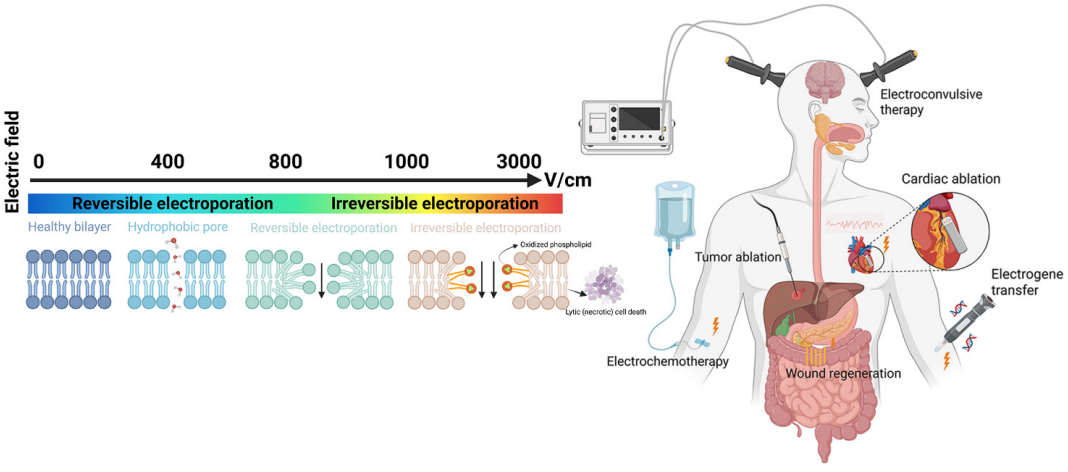


Figure 1 Overview of reversible and irreversible electroporation techniques and *in vivo* applications. The illustration was created using BioRender.

2.3 Inflammatory and immune responses

IVE typically induces localized inflammatory and immune responses, crucially influencing both therapeutic outcomes and tissue recovery [47, 48]. These responses can manifest as transient tissue swelling, localized inflammation, and recruitment of immune cells such as neutrophils, macrophages, and lymphocytes [49–52]. Moderate inflammation may enhance therapeutic efficacy, particularly in immunotherapy applications, by promoting antigen presentation and amplifying local immune activation [53]. Conversely, excessive inflammatory responses may result in collateral tissue damage, prolonged healing times, or patient discomfort, requiring careful management through parameter optimization or adjunctive anti-inflammatory interventions [54].

The immune response during electroporation therapy can be precisely controlled by adjusting pulse duration and type. Studies have shown that nanosecond pulses induce the strongest damage associated molecular patterns (DAMPs) release and the most significant immune response, while millisecond pulses induce the weakest effect. By optimizing pulse parameters, it is hoped that unnecessary inflammatory responses can be mitigated without compromising treatment efficiency, thereby improving treatment safety and effectiveness [45]. Understanding these immune interactions allows clinicians and researchers to harness beneficial inflammatory responses, such as those enhancing immune-stimulatory effects in vaccine delivery, while minimizing adverse side effects [55].

2.4 Electrode design and placement strategy

Electrodes influence electric field distribution, penetration depth, and spatial uniformity, directly affecting treatment outcomes and safety [56, 57]. Thus the electrode design, geometry, and precise placement are critical in achieving effective, targeted IVE. Recent innovations of electrode design include needle, plate, microneedle, and flexible electrodes, each suited for distinct clinical applications. For instance, needle electrodes deliver focused electric fields to deep tissue targets, whereas flexible and conformal electrodes adapt to anatomically irregular surfaces, providing uniform field distribution despite physiological movements [58].

Electrode placement, even a few millimeters of deviation, can result in insufficient electric field strength in the tumor which is located near major hepatic blood vessels, bile ducts, and other heat-sensitive structures, thus affecting the integrity and effectiveness of IRE treatment [39, 59]. Moreover, due to the heterogeneous conductivity and complex anatomical structure of biological tissues such as the liver, pancreas, and brain, the actual electric field distribution *in vivo* often deviates from theoretical expectations, making standardized electrode placement particularly difficult [21]. To address this challenge, precise electrode positioning guided by imaging techniques such as ultrasound or magnetic resonance imaging (MRI) has been shown to significantly improve treatment accuracy and reduce inter-individual variability during surgery and variability in treatment outcomes [56, 60]. Real-time monitoring through impedance measurements or imaging allows adaptive adjustments of pulse parameters mid-treatment, ensuring optimized electroporation conditions [56]. Computational modeling further aids electrode placement strategies by simulating tissue-specific electrical properties, providing predictive insights into field distribution and therapeutic efficacy [61].

Although IRE is primarily considered a non-thermal ablation technique, localized Joule heating inevitably occurs due to tissue resistivity and pulse repetition, which can transiently raise tissue temperatures near the electrodes. This mild temperature elevation (typically < 50 °C) may enhance electroporation efficiency by increasing lipid bilayer fluidity and lowering the transmembrane voltage threshold required for pore formation. However, excessive or cumulative heating (> 60 °C) can shift the mechanism from non-thermal electroporation to thermal coagulative necrosis, leading to unintended injury of adjacent heat-sensitive structures such as bile ducts or blood vessels [62]. Therefore, accurate thermal monitoring and modeling have become essential in IRE planning, ensuring that the ablation zone remains within a physiologically safe temperature window. Recent studies emphasize that the spatial distribution of temperature rise is highly heterogeneous, and even minimal heat-sink effects from local perfusion or major vessels can significantly alter the effective ablation zone and therapeutic predictability [63, 64].

2.5 Computational modeling and real-time monitoring

IRE effectively avoids the risk of thermal damage. This makes it an ideal choice for managing malignant liver tumors in anatomically challenging locations. Computational modeling further enhances the application advantages of IRE by accurately simulating the electric field distribution within biological tissues [65, 66]. It is often based on finite element analysis (FEA) or computational fluid dynamics (CFD), incorporate key physiological factors such as

anatomical variability, heterogeneous tissue conductivity, and anisotropic properties [67, 68]. Knabben M. A. et al. developed an empirical model to capture the dynamic behavior of electroporation. They implemented this model within a MATLAB®-based finite element framework, demonstrating its effectiveness as a robust tool for simulating electroporation phenomena [69].

In clinical applications, in addition to using computational models to predict electric field distribution, for the ablation of heat-sensitive and anatomically complex liver tumors, combining nonlinear electrical models with clinical imaging technology can provide key insights into the electric field distribution under real treatment conditions, thereby providing strong support for the optimization of treatment strategies [70]. This combination of modeling and imaging not only enhances simulation accuracy but also bridges the gap between theoretical models and clinical implementation. Importantly, these advanced models empower clinicians to personalize electrode placement, pulse parameters, and treatment protocols based on individual patient anatomy and pathology, ultimately improving treatment efficacy and outcome predictability [71].

Real-time impedance monitoring serves as a vital complement to computational modeling by providing dynamic, intraoperative feedback during electroporation procedures [72, 73]. Fluctuations in impedance reflect changes in tissue properties or the successful occurrence of electroporation, enabling clinicians to fine-tune parameters such as pulse duration, frequency, and amplitude in real time [74]. In addition, real-time impedance monitoring enables continuous assessment of tissue temperature at the treatment site, which is particularly important for monitoring electrode temperature near or in direct contact with anatomically sensitive structures that are susceptible to thermal injury [75, 76]. Such monitoring is crucial for ensuring the safety of the procedure. For example, in hepatic applications, thermal damage to the bile ducts can result in life-threatening complications [76]. Expanding upon this concept, Campelo S. and colleagues developed a data-driven state-space model to assess the feasibility of real-time electrode temperature monitoring based on impedance measurements. They got high model accuracy by using agar phantoms to mimick various tissue conductivities and voltage conditions, which was further validated through finite element simulations. These findings confirm that impedance data can be used not only to monitor electroporation efficiency but also to estimate temperature changes during treatment [77]. When integrated with automated, algorithm-driven control systems, real-time monitoring technologies can significantly enhance procedural precision, consistency, and overall patient safety in clinical settings.

Effective implementation of IVE in clinical and therapeutic settings demands an in-depth understanding of underlying molecular mechanisms, as well as consideration of tissue-level and physiological complexities inherent to *in vivo* environments [39, 44, 48]. Comprehensive knowledge of lipid membrane dynamics, pore formation kinetics, tissue-specific conductivity, immune responses, electrode design, and advanced monitoring methodologies is vital for refining electroporation protocols.

3 Structure and types of IVE devices

The design of IVE devices plays a critical role in ensuring the efficacy, safety, and adaptability of molecular delivery across a range of biomedical and therapeutic applications. A standard electroporation system generally comprises four core components: a high-voltage pulse generator, an electrode system, a digital control module, and an injection device (Fig. 2(a)) [88, 89]. The pulse generator provides the electric field necessary for membrane permeabilization, while the electrodes deliver these pulses directly to the target tissue [90]. By optimizing electrode geometry and configuration, needle arrays, plate electrodes, and flexible microelectrodes have improved delivery accuracy and minimized invasiveness [9]. Meanwhile, digital control modules can enhance the precision of pulse timing, duration, and amplitude, thus increasing delivery consistency and reliability. Injection modules ensure the effective delivery of therapeutic agents [9, 86]. To support clinical translation, portable and programmable pulse generators have been developed, allowing flexible use in both research and clinical settings [91]. A summary of various types of electroporation electrodes and their corresponding electrical parameter settings is shown in Table 1. These advancements have significantly expanded the applicability of electroporation in areas such as oncology, regenerative medicine, vaccine delivery, and targeted immunotherapy, offering a scalable platform for next-generation therapeutic delivery [92].

3.1 Pulse generators

At the heart of the system lies the pulse generator, which governs the electrical stimulation required for either molecular transport or tissue ablation [93, 94]. Advanced pulse generators now support a range of waveform modes, allowing for greater adaptability across therapeutic applications. Unipolar pulses, characterized by their short duration and low voltage, are commonly employed in gene therapy due to their ability to enhance nucleic acid uptake while minimizing tissue disruption [95, 96]. In contrast, bipolar pulses, which alternate polarity, are better suited for tumor ablation, as they help reduce muscle contractions and patient discomfort during high-voltage exposure [97–99]. To further improve treatment precision, research-grade electroporation systems are increasingly equipped with impedance feedback algorithms, enabling real-time modulation of pulse parameters during the procedure [100]. This closed-loop control system enhances clinical consistency, ensures treatment safety, and improves adaptability to individual patient needs. Besides waveform selection, the pulsing strategy of using single or multiple pulses must also be carefully coordinated with the treatment goals and the specific characteristics of the target tissue. Multi-pulse protocols, involving repeated delivery of lower-intensity pulses, are particularly effective in enhancing molecular transfer. These regimens promote membrane permeabilization, facilitating greater intracellular uptake while minimizing irreversible damage [89]. Gehl et al. demonstrated that multi-pulse strategies significantly improve gene delivery efficiency, especially in skeletal muscle tissues [101]. Furthermore, recent advances have shown their utility in enhancing the delivery of CRISPR-Cas9 constructs for precise genome editing [102]. In contrast, for interventions that require rapid and instantaneous cell permeabilization, such as cardiac ablation, single high-intensity pulses are typically preferred. This approach offers several advantages, including the mitigation of thermal effects, improved spatial precision, and minimal collateral tissue damage, all of which are critical for ensuring procedural safety and efficacy [103].

In addition to the waveform parameters， modern pulse generators offer other precisely adjustable parameters, including amplitude, number, duration and frequency, allowing clinicians to

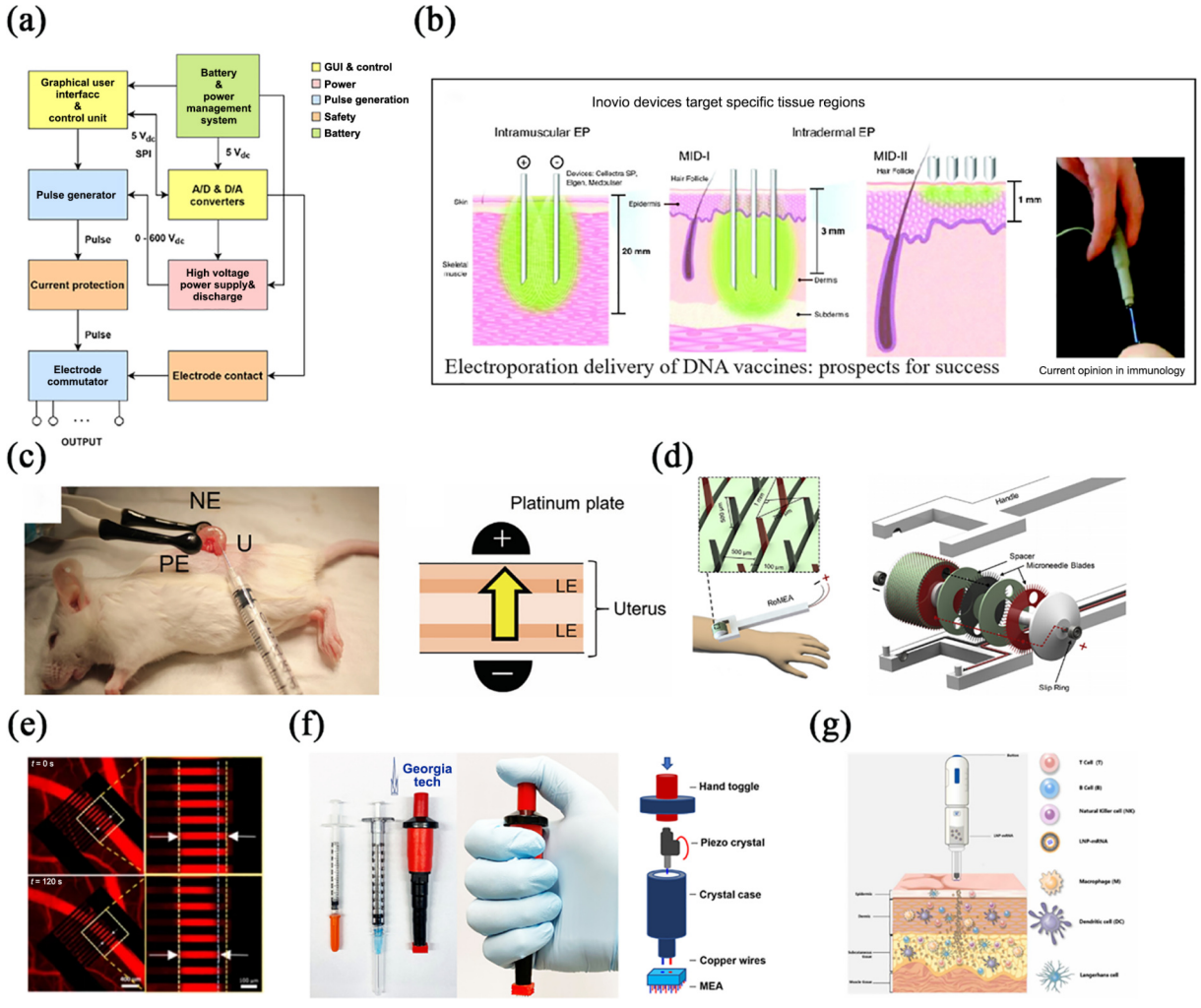


Figure 2 (a) The main components of the electroporation device [89]. (b) Electroporation devices developed to target the different depths of the skin/muscle. A non-contact device where EP is facilitated by piezoelectric discharge (PID) is also shown [113]. Reproduced with permission from Ref. [113], © Elsevier Ltd. All rights reserved 2011. (c) Schematic of *in vivo* electroporation using platinum plate electrodes: Experimental setup (left); illustration of the electric current direction (yellow arrows, right). PE: positive electrode; NE: negative electrode; U: uterus; LE: luminal epithelium [141]. Reproduced with permission from Ref. [141], © by the Japanese Society of Veterinary Science 2017. (d) Schematic diagram and operation diagram of the minimally invasive RoMEA continuous electroporation targeting tissue device [154]. Reproduced with permission from Ref. [154], © Elsevier Ltd. All rights reserved 2020. (e) Integrating flexible electronics for pulsed electric field delivery in a vascularized 3D glioblastoma model [170]. Reproduced with permission from Ref. [170], © Lefevre, M. C. et al. 2021. (f) The left panel shows the comparison of ePatch with 0.5 and 1 mL syringes, and the right panel shows the components of ePatch [179]. Reproduced with permission from Ref. [179], © Xia, D. et al. Published by PNAS 2021. (g) A needle-free syringe was used to subcutaneously inject the mRNA-LNP model [180]. Reproduced with permission from Ref. [180], © Mao, S. et al. Published by Elsevier Ltd 2022.

Table 1 List of various types of electroporation electrodes and corresponding electrical parameters[a]

Field of application	Electrode type	Power supply	Applied voltage type	Magnitude	Ref.
Nonviral gene transfer device	Plate electrode	Pulse generator	Pulsed electric field	10–100 V	[78]
Developmental biologyelectroporation	Wire electrode	DC power source	Square-wave pulses	50–200 V	[79]
Skeletal muscle gene delivery	Needle electrode	Pulse generator	Exponential decay pulses	100–300 V	[80]
Cancer immunotherapy electroporation	Plate electrode	DC power source	High-voltage pulses	100–250 V	[81]
Microfluidic electroporation	Needle array	Microfluidic power supply	Continuous pulses	20–80 V	[82]
Microneedle electroporation	Microneedle array	Low-voltage power supply	Square-wave pulses	30–50 V	[83]
DNA vaccine delivery	Plate electrode	DC power source	Pulsed electric field	200–400 V	[84]
Retinal gene transfer	Wire electrode	AC power source	Sinusoidal pulses	100–250 V	[85]
Neural development electroporation	Plate electrode	Pulse generator	Biphasic pulses	50–200 V	[86]
Mucosal DNA vaccine electroporation	Wire electrode	DC power supply	High-voltage pulses	150–300 V	[87]

[a]DC: direct current; AC: alternating current.

tailor treatments to specific therapeutic and anatomical requirements [104]. Platforms such as SmartGene and CELLECTRA exemplify this sophistication, offering programmable, user-friendly interfaces with real-time adaptability [105, 106]. For example, to address the challenge of heterogeneity commonly observed in clinical tumors, Douglas W. et al. developed an electroporation generator capable of implementing feedback control based on impedance spectroscopy and equivalent circuit model parameter estimation. This system enables real-time evaluation of the electrochemical properties of tissues. By employing a feedback-based electroporation strategy, the device achieves maximal expression of reporter genes while significantly reducing variability and the required applied energy. These results represent a meaningful advancement in the field of gene electrotransfer [107]. Technological innovation has also improved hardware architecture, with modern pulse generators incorporating microcontroller-based circuitry. Digital feedback loops and power optimization strategies enable portable or battery-powered devices to maintain pulse stability across varying battery levels and environmental conditions [108]. This ensures precise and reproducible output, leading to uniform treatment outcomes and thereby enhancing the accessibility of electroporation technology in both clinical and remote settings [109, 110].

However, achieving optimal therapeutic outcomes requires more than just refining electrical parameters. It also calls for careful synchronization between pulse design and electrode architecture. Electrode configuration plays a pivotal role in shaping the electric field distribution, determining the depth of penetration, and influencing the extent of tissue interaction. Therefore, the co-optimization of pulse delivery settings, such as waveform, frequency, and duration, alongside electrode geometry forms the foundation of modern IVE systems. Therefore, the progress made with different electrodes will be discussed in the following sections.

3.2 Needle electrodes

Invasive needle electrodes are designed with sharp tips and variable lengths to penetrate tissue directly, enabling the delivery of electrical pulses deep into the target tissue with high spatial precisions [111, 112]. This precision makes them particularly advantageous for treating dense anatomical structures, such as solid tumors. Needle electrodes are also the most widely used invasive devices for targeting deep tissues, with various designs developed to accommodate different skin and muscle depths (Fig. 2(b)) [113].

The ability to generate highly focused electric fields even with a single needle electrode further highlights the precision and tissue protection capabilities of this invasive technology [114]. For example, the NanoKnife system is an advanced research device that has been approved by the U. S. Food and Drug Administration (FDA) and clinically authorized. It uses a monopolar needle electrode during tumor ablation [115, 116]. These electrodes often contain an echogenic coating that facilitates real-time ultrasound-guided placement, ensuring accurate positioning and minimizing damage to adjacent tissues [117]. To improve treatment efficacy and field uniformity, adjustable multi-needle arrays are often used. These arrays can be adjusted in number, spacing, and arrangement according to the size and morphology of the tumor and are usually located around the lesion [59, 118–120]. In addition, the adjustability of needle length and electrode spacing enables clinicians to tailor the treatment area to lesions of varying sizes and shapes, thereby enhancing clinical versatility and adaptability [59]. Needle electrodes are commonly available in monopolar, bipolar, and multipolar configurations, enabling precise targeting of tissues such as solid tumors, muscle, liver, and pancreas [121–124]. A key advantage of needle-based systems is the ability to precisely control the spatial characteristics of the electric field by applying pulses between selected needle pairs. This selectivity significantly reduces off-target effects and minimizes collateral damage to surrounding tissues [125–127]. For example, concentric needle electrodes can selectively activate different electrode pairs to optimize electroporation outcomes [128].

Recent innovations in needle electrode technology include an insulating rod coating with an exposed tip that precisely confines the electric field to the target site, further reducing the risk of damage to surrounding tissue and muscle contraction during electroporation [129]. Advances in electrode tip design driven by precision manufacturing techniques have also reduced insertion forces and improved patient comfort. Furthermore, the development of sophisticated multi-electrode arrays has enabled the creation of complex, patient-specific electric field patterns. These innovations support a range of therapeutic goals, from precise tumor ablation to controlled gene delivery, and expand the application of needle electrodes in different clinical scenarios.

3.3 Plate electrodes

Plate electrodes are mainly used for non-invasive or superficial electroporation because they can achieve a wide and uniform surface electric field distribution. To address the challenge of high resistance in the stratum corneum, the design of such electrodes usually introduces conductive gels or special surface coatings to reduce skin resistance and improve electrical contact performance [130, 131]. Plate electrodes perform particularly well in skin-related applications, such as DNA vaccine delivery, where their large electric field coverage allows efficient transfection of antigen-presenting cells in the epidermis and dermis [132]. To reduce patient discomfort and the risk of thermal injury, pulse parameters, including waveform, duration, and voltage amplitude, need to be precisely controlled and optimized. Non-invasive electroporation is usually achieved by placing plate electrodes directly on the skin or tissue surface, avoiding the puncture pain and related discomfort caused by traditional invasive needle electrodes [133].

Plate electrodes used for surface electroporation have a variety of types, including parallel plate, caliper, tweezers, and L-shaped designs [133], and are usually made of highly conductive and biocompatible materials such as brass, stainless steel, platinum, or gold-plated metals [134, 135]. Among the various types, flat electrodes are the most common and widely used type for non-invasive treatment [136, 137]. This type of electrode is usually composed of two rectangular stainless-steel plates with a fixed or adjustable spacing between the plates [138]. The electric field is mainly concentrated in the edge area of the electrode. This feature is conducive to local electroporation, thereby significantly improving the delivery efficiency of surface drugs and genes [139, 140]. For example, Kobayashi et al. developed a novel and efficient mouse uterine gene transduction technology using a plate electrode for *in vivo* electroporation. This method can achieve highly localized gene expression and precisely confine transgenic activity to the luminal epithelial cells at the location of the positive plate, thus providing the possibility to study the molecular function of specific sites (Fig. 2(c)) [141]. Flat electrodes have a simple structure and are easy to operate. Their geometric configuration

facilitates the generation of a uniform electric field on the tissue surface, making them particularly suitable for the treatment of diseases on the surface or shallow layer of the skin [142]. Tweezer electrodes are a variant of parallel plate electrodes. The advantages of forceps-type plate electrodes lie in their compactness and ease of use, allowing for tight and precise gripping of target tissues, making them highly adaptable in clinical scenarios that require precise positioning [57]. This type of electrode is particularly suitable for the treatment of tumors located on the surface of the skin or in shallow tissues, and shows good therapeutic prospects in exophytic tumors, melanoma, and certain specific scenarios (such as in utero or *ex vivo* gene transfection [134, 143]. L-shaped electrodes are another commonly used structure and are widely used in veterinary medicine and preclinical research. They can be effectively used to treat diseases such as squamous cell carcinoma in small animals, equine sarcoma, and melanoma. In addition, L-shaped electrodes also show unique advantages in the treatment of "contaminated edges" of surgical fields after mast cell tumor or soft tissue sarcoma resection [144]. Mazères et al. have verified the practicality of L-shaped electrodes in *in vivo* gene therapy and electrochemical therapy. In their study, rotating the electrodes 90° after each pulse sequence effectively compensated for electric field inhomogeneities, resulting in a complete remission rate of 93% in treated animals. The approach was effective for tumors up to 5 cm in diameter and enabled efficient gene delivery through 4 mm gap electrodes [58, 142].

The significant advantage of plate electrodes is that they have good repeatability and can be directly attached to the skin surface for rapid adjustment, avoiding invasive operations such as needle electrodes, thereby significantly reducing the discomfort and risk of trauma to patients during treatment. This non-invasive feature makes it safe and easy to operate in clinical applications. However, since the electrodes are mainly placed on the surface of the affected area, the limitation of the limited penetration depth of the electric field is also prominent. This problem is mainly due to the high resistance characteristics of the stratum corneum of the skin, which causes the voltage to decay rapidly in the epidermis and the electric field is difficult to effectively act on the deep tissue [95]. Therefore, plate electrodes are effective in the treatment of skin diseases or superficial tissue lesions, but their applicability in the treatment of deep tissues is still significantly limited. To overcome the limitation, current research is actively exploring various strategies to improve the penetration and depth of the electric field, aiming to promote the application and transformation of non-invasive electroporation technology in a wider range of clinical scenarios [145]. For example, researchers have proposed using methods such as optimized electrode layout, pulse parameter control, enhanced electrode materials, magnetoelectric nanoparticles, light, heat, and ultrasound energy assistance to improve the uniformity and penetration depth of the electric field within the tissue, thereby achieving precise treatment of deep tissues, aiming to promote the application and transformation of non-invasive electroporation technology in a wider range of clinical scenarios [146–148].

In recent years, the design of plate electrodes has also undergone important technological innovations. Advanced processes such as the use of flexible conductive materials and integrated hydrogel interfaces not only significantly improve the conformability of the electrode, but also effectively reduce the interfacial impedance between the tissue and the electrode [149]. These improvements significantly improve the comfort and electrical transfer efficiency during treatment, thereby enhancing patient compliance with long-term and repeated treatment plans. In addition, modern plate electrodes can also integrate temperature-sensitive sensors to achieve real-time monitoring of thermal effects during treatment, thereby further ensuring treatment safety and improving the overall comfort experience of patients [74].

3.4 Microneedle arrays

The research on *in vivo* electroporation electrodes now has broken through the design paradigm of traditional macroscopic electrodes and moved towards a more forward-looking direction. Current technological progress is mainly focused on the miniaturization of electrodes, the functional upgrading of materials and the high integration with flexible devices. To further improve patient comfort and enhance the accuracy of operation, electrode design is accelerating towards the micrometer and even nanometer scale.

Emerging as minimally invasive alternatives, microneedle arrays combine the precision of needle electrodes with reduced invasiveness, making them particularly suitable for intradermal delivery [150]. The high-density microneedle array (HIDMA) developed by O'Mahony et al. realizes a low-voltage, low-damage *in vivo* electroporation gene delivery strategy. Through a hexagonal arrangement and a three-pole connection structure, the system uses micron-level needle spacing and tens of volts of voltage to effectively penetrate the epidermis, reduce contact impedance, and significantly improve DNA plasmid transfection efficiency and reduce cell damage [151]. These microneedle arrays are made of silicon, stainless steel, or soluble polymers prepared by micro-molding technology, which can achieve precise control of penetration depth and electrode spacing [152, 153]. They significantly lower required voltages, thereby reducing patient discomfort without compromising transfection efficiency. For example, Huang et al. proposed a new design that integrates microneedles and electrodes. They used planar laser micromachining technology to fabricate a rolling stainless steel microneedle electrode array (RoMEA) with microneedles on the edge, which can achieve large-area, minimally invasive electroporation of DNA on the skin surface (Fig. 2(d)) [154]. This device has the advantages of low trauma, good biocompatibility, easy operation and low cost, providing a promising clinical solution for skin disease treatment and intradermal vaccination. Another significant advancement in the development of microneedle electrodes is their integration with flexible electroporation technologies, which has emerged as a pivotal direction in transdermal gene delivery research. Two illustrative examples exemplify this progress, both demonstrating the synergistic combination of microneedles and flexible electrodes to enhance skin electroporation efficacy. One strategy involves the coupling of a microneedle roller with a flexible interdigitated electrode array (FIEA), facilitating efficient delivery of DNA and siRNA to murine skin. This approach exhibits superior nucleic acid transfer efficiency and improved safety profiles at lower voltages compared to conventional electroporation alone [155]. Concurrently, another study reports a minimally invasive *in vivo* electroporation technique that employs xylene-based flexible electrodes following microneedle pretreatment. This method establishes a sufficient subcutaneous electric field at low voltages, with enhanced field uniformity and biocompatibility contributing to elevated transfection rates and diminished skin damage. Furthermore, its cost-effectiveness and operational simplicity render it a promising candidate for clinical

applications [156]. In summary, from the application of new materials to the ingenious design of integrated equipment, the development of microneedle electroporation systems has made great progress, laying a solid foundation for its clinical transformation. It is these technological innovations that have brought multiple advantages such as low voltage, high efficiency, less trauma and convenient operation, thus paving the way for practical application. Finally, in the clinical trials of DNA vaccination, it showed extremely high patient acceptance and strong immune response, clearly revealing its broad prospects as a next-generation gene delivery platform [112, 157].

The integration of biodegradable materials with conductive functionalities represents a promising strategy for achieving safer and more precise transdermal delivery. Recently, biodegradable polymeric microneedles, fabricated through advanced micro-molding or additive manufacturing techniques, are engineered to dissolve safely following administration. This eliminates the risk of needle retention or the need for repeated insertions, thereby enhancing safety and significantly improving patient compliance [158]. These dissolving systems offer an excellent balance of mechanical strength and biocompatibility while minimizing long-term tissue irritation [159]. In parallel, 3D printing technologies are being increasingly adopted to fabricate conductive microneedles, offering significant advantages over traditional micro-electro-mechanical system (MEMS)-based fabrication. Unlike complex photolithographic or etching processes, 3D printing enables customizable geometries, rapid prototyping, and cost-effective manufacturing, which are critical for scalable biomedical applications [160]. These approaches now allow excellent precision in controlling electrode geometry and spacing, optimizing electric field distribution and enhancing therapeutic outcomes in electroporation-based gene therapy [161].

Innovations also have demonstrated that combining flexible biodegradable substrates with highly conductive materials like polypyrrole, gold, or graphene leads to multifunctional devices that support electroporation, sensing, and drug delivery in a single platform [162, 163]. These multifunctional microneedles can deliver DNA, siRNA, and therapeutic proteins directly into the dermis with minimal voltage and minimal tissue disruption, thus holding strong promise for applications in clinical medicine [164].

3.5 Flexible electrodes

Flexible electrodes can conform to the anatomical structure of irregular or dynamic biological surfaces such as the heart, gastrointestinal tract, and neural tissue, and maintain stable contact and uniform electric field distribution during continuous movement [165, 166]. These micro-nano electrodes are often made using advanced micro-nano manufacturing technologies and are compatible with a variety of functional materials, such as conductive polymers (PEDOT), gold, carbon nanotubes, and nickel-titanium (Ni-Ti) shape memory alloys, to achieve highly localized energy delivery [167]. In a representative development, Kim et al. developed a new self-expanding electrode based on Ni-Ti memory alloy wire, which can be used for electroporation treatment of hollow organs such as blood vessels and intestines, and has the ability of adaptive expansion and precise energy focusing [168]. The same team also proposed a two-finger flexible electrode and significantly reduced the electrode impedance through the platinum black electrodeposition process, achieving effective electroporation at a voltage of only 50 V, and synchronously monitoring tissue impedance changes to perform online feedback evaluation of the ablation effect. The electrode system has been successfully integrated with a balloon catheter and has good clinical transformation prospects [168]. Flexible implantable electrodes have shown significant advantages in multiple medical fields, especially in the treatment of neurological diseases. For example, they can be used for precise ablation of epileptic foci and effectively protect surrounding healthy tissues [169]; in tumor treatment, such as vascularized 3D glioblastoma model, flexible electrode platforms promote local drug delivery and treatment response, improving efficacy (Fig. 2(e)) [170]; and in cardiac rhythm regulation, such devices can intervene in arrhythmias through precise neural regulation to avoid secondary damage to the myocardium [171].

Flexible microelectrode technology has made significant progress recently, and its applications are rapidly expanding. These applications include critical frontier research areas such as low-voltage precise gene delivery and high-fidelity bioelectrical signal monitoring. The teams of Professors Chang and Li have developed a flexible nanofluidic (NanoFLUID) patch that safely delivers payloads into target organ cells via electroporation at a low voltage of 20 V. This approach enhances intracellular transport by approximately 1×10^5-fold compared to conventional diffusion. *In vivo* studies demonstrated the platform's high efficacy and safety in cancer therapy, damage repair, and genetic screening, establishing it as an innovative bioelectronic tool for therapeutic and research applications [172]. It is noteworthy that the team has also extended its technology to more challenging *in vivo* healing contexts. Intestinal wound healing remains a significant clinical challenge, often complicated by postoperative issues associated with traditional suturing. To overcome this, the team designed a self-powered, soft, and fully biodegradable electronic bandage that promotes intestinal wound repair. The device utilizes a dual electrostimulation strategy: a pulsed current facilitates electrotransfection of epithelial cells for targeted gene expression, while a direct current stimulates the secretion of healing factors, collectively accelerating wound recovery [173]. In the field of neuroscience, flexible microelectrode technology also shows its unique advantages. The flexible microelectrode array (FERE) proposed by Zhang et al. successfully overcomes the problems of high voltage, low positioning accuracy and difficult operation in traditional retinal electroporation. Through flexible structural design and precise electric field control, the system achieves layered gene delivery to different retinal tissue layers at only 5 V voltage, verifies the controllable transfection ability for the first time, and marks a key step towards clinical practical application of retinal electroporation technology [174]. The electric field-driven 3D printed flexible nanosilver electrode array (FlexNEA) developed by Shi et al. provides a new high-performance platform for cardiac electrophysiology research. The device can be quickly prepared within minutes and achieves an intracellular recording success rate of more than 99% through efficient electroporation. Its flexible structure enhances the close coupling between cardiomyocytes and electrodes, significantly improving signal quality and stability. More importantly, the sensitive response ability of FlexNEA in drug screening provides an accurate and quantitative tool for ion channel pharmacology research, and promotes the widespread application of low-cost, biocompatible microelectrode systems in preclinical cardiovascular research [175].

3.6 Imaging-guided and real-time monitoring electrodes

In terms of preoperative planning, advanced systems often use computed tomography (CT) or MRI scan data for personalized three-dimensional modeling to accurately design electrode layout. During surgery, the instant feedback obtained through impedance monitoring can be used to adjust pulse parameters in real time to ensure the consistency of tissue permeability, further improving efficacy and safety. This fusion mode of "real-time integration of loading feedback control" is becoming an important development direction for high-precision electroporation treatment [10]. At the microscopic scale, imaging methods such as total internal reflection fluorescence microscopy and digital holographic microscopy have been used to directly observe the electroporation process at the cellular level and the physiological changes induced by it [176]. In addition, positron emission tomography (PET), especially prostate-specific membrane antigen (PSMA) PET-CT, has also been used for enhanced target identification in prostate cancer treatment [177]. Simultaneously, the integration of optical imaging technology and optogenetics is providing more precise spatiotemporal control and functional dynamic monitoring methods for electroporation, expanding its application boundaries in cutting-edge fields such as neural regulation and cell manipulation [178]. Imaging fusion and multimodal feedback systems are becoming key development directions for personalized electroporation therapy in the future.

3.7 Portable and mobile IVE systems

In recent years, significant progress also has been made in portable electroporation technology, which has made it possible to apply IVE therapy in outpatient clinics and remote areas. This type of battery-powered compact system not only maintains the required high-voltage delivery capacity, but also significantly enhances operational flexibility and field adaptability. Lightweight handheld electroporation devices are particularly suitable for treatment in resource-limited or field environments, thereby expanding the accessibility of cancer treatment and vaccination programs worldwide.

With the development of energy-saving circuits and modular designs, these devices can be flexibly adapted to a variety of electrode configurations, further improving clinical compatibility and user-friendliness. For example, a variety of low-cost, easy-to-operate portable devices have been developed for DNA vaccination and point-of-care diagnostic applications, such as ePatch and ElectroPen [10]. Xia et al. designed an ultra-low-cost (< $1), lightweight (< 50 g), battery-free handheld electroporation system. The system combines a thumb-driven piezoelectric pulse generator with a microneedle electrode array skin interface for COVID-19 DNA vaccination (Fig. 2(f)). Animal experiments have shown that the device has good immunogenicity and tolerability [179].

In addition, with the continuous evolution of technology, needle-free syringes have also been introduced into the electroporation vaccination system. This type of device not only significantly reduces injection pain but also shows stronger immunogenicity in animal models (Fig. 2(g)) [180]. Studies have further found that needle-free injection can activate the innate immune response of the skin and play a role similar to that of a physical adjuvant. Saed Abbasi et al. successfully delivered a naked mRNA SARS-CoV-2 vaccine using this method, effectively limiting its local distribution and avoiding systemic proinflammatory responses [181]. Looking ahead, this type of non-invasive innovative design is expected to further inspire the development of a new generation of handheld electroporation devices. Intelligent programmable electroporation generators equipped with a user-friendly interface, customizable parameters, and remote networking capabilities will provide stronger technical support for standardized treatment processes, quality control, and personalized medicine.

3.8 Closed-loop and automated systems

A prominent direction in the advancement of IVE technology is the integration of closed-loop feedback systems and automation, increasingly powered by AI and machine learning algorithms. These intelligent platforms are capable of analyzing real-time bioelectrical signals such as tissue impedance, thermal responses, and electrochemical feedback, to dynamically adjust pulse parameters, thereby enhancing delivery efficiency and procedural precision [107].

Such closed-loop system with feedback module significantly reduces variability stemming from operator-dependent factors, standardizes treatment administration, and allows personalized modulation of pulse settings in response to each patient's specific tissue characteristics. Importantly, AI-assisted electroporation systems have been shown to optimize field distribution while preventing overtreatment and minimizing cellular damage, particularly in tumor ablation and gene therapy contexts [182]. Additionally, automated platforms equipped with temperature sensors, electrical probes, and adaptive algorithms are under development to achieve real-time correction of field asymmetry or electrode misplacement. These systems can automatically regulate treatment duration and waveform selection, ensuring treatment uniformity even in anatomically complex or moving tissue [183].

Recent studies further demonstrate the use of deep learning frameworks to predict patient-specific electroporation outcomes based on pre-treatment imaging and physiological data, leading to precision-guided gene delivery [153]. These approaches are crucial for enabling self-driving electroporation systems capable of closed-loop operation in minimally invasive or even wearable therapeutic settings.

Overall, the convergence of AI, real-time biofeedback, and autonomous control is transforming IVE into a smart, adaptive platform-poised to meet the demands of precision medicine and expand its role in next-generation therapeutic protocols.

4 Therapeutic applications of IVE

As mentioned above, with the continuous advancement of IVE device design, the technology is undergoing a rapid evolution from traditional invasive electrodes to minimally invasive or even non-invasive systems. This innovation has significantly improved the safety, efficiency and repeatability of the operation, while greatly expanding the types and application scenarios of bioactive molecules that can be delivered [184–186]. Through these technological upgrades, the potential of IVE is continuously released in multiple medical fields. It can effectively deliver a variety of macromolecules or small molecules to target tissue cells, including but not limited to nucleic acids (such as DNA, mRNA, siRNA), proteins and enzymes, small molecule compounds, viral vectors, etc.

This type of locally controllable delivery strategy not only greatly improves the efficiency of cell uptake, but also minimizes systemic side effects and immunogenicity risks, ensuring efficacy while enhancing safety [187, 188]. Table 2 systematically classifies the types of biological molecules currently delivered through IVE and

Table 2 Summary of types of molecules delivered by electroporation and their location of application

Types of molecules delivered	Form of electroporation	Location of application	Type of disease applied	Ref.
Plasmid/DNA	*In vivo* electroporation	Skeletal muscle	Muscular disorders	[80]
	Focal electroporation	Adult mouse brain	Neurological research	[189]
	Electroporation for gene therapy	Various tissues	Cancer, infectious diseases	[188]
	Electroporation with hyaluronidase	Skeletal muscle	Gene therapy	[190]
siRNA	Microneedle array electroporation	Skin	Gene therapy	[83]
	Electroporation patch	Tumors, muscle	Cancer gene therapy	[191]
	Electroporation-based intracellular delivery	Various tissues	Gene therapy, cell reprogramming	[192]
Various molecules	On-chip electroporation	Microalgae	Bioengineering	[193]
	Progressive electroporation	Intracellular	Cell engineering	[192]
	Soft electroporation	Mammalian cells	Drug delivery	[169]

their main targets, demonstrating its technical adaptability and functional diversity.

A particularly notable application of IVE lies in transdermal drug delivery (TDD). TDD is inherently non-invasive and advantageous in bypassing hepatic first-pass metabolism; however, its efficacy is largely constrained by the stratum corneum (SC), which constitutes a formidable barrier to most hydrophilic and high-molecular-weight compounds. In this context, IVE emerges as a highly effective physical enhancement strategy. By applying brief, high-voltage electric pulses, IVE transiently induces the formation of nanoscale aqueous pores within the lipid bilayers of the SC, thereby reducing skin impedance and facilitating the electrophoretic transport of charged molecules across the barrier. This technique enables the transdermal delivery of a broad spectrum of therapeutic agents, from small molecules (< 500 Da) to complex macromolecules. Notably, it allows for the efficient administration of therapeutic nucleic acids and proteins — such as DNA vaccines and insulin — into underlying antigen-presenting cells (e.g., dendritic cells) or directly into systemic circulation. Moreover, recent integration of IVE with microneedle electrode arrays has further improved delivery precision and efficiency, paving the way for painless, self-administered therapeutic regimens. This shift toward transdermal delivery benefits from both a deeper understanding of cutaneous immunology and technological innovations.

This section will further classify and analyze the types of molecules that can be delivered by electroporation and focus on its potential clinical value in emerging fields such as personalized treatment and translational medicine.

4.1 Gene therapy and genome editing

Electroporation is often performed by injecting nucleic acids subcutaneously, intramuscularly, or intradermally, and then applying a local electric field to induce transient permeability of the cell membrane and promote the entry of nucleic acids into cells [194, 195]. It is reported that its DNA uptake can be hundreds to thousands of times higher than passive diffusion [196, 197]. The delivery of exogenous vascular endothelial growth factor (VEGF) to ischemic areas has demonstrated significant potential in promoting therapeutic angiogenesis. For instance, Ferraro and Cruz effectively delivered plasmid DNA encoding VEGF165 (pVEGF) into a preclinical ischemic wound model (pVEGFE$^+$) via intradermal injection combined with electroporation, which significantly enhanced blood perfusion and wound healing [198]; Dezawa et al. also successfully delivered genes to retinal ganglion cells, achieving a green fluorescent protein (GFP) gene expression rate of more than 40% [199, 200]. In addition to DNA, electroporation is also widely used for the delivery of mRNA. mRNA can directly express therapeutic proteins or antigens in the cytoplasm, avoiding the limitation of entering the nucleus. Although naked mRNA is usually less efficient in uptake, the formation of transient pores on the cell membrane with the help of electroporation can significantly improve its delivery efficiency [46]. Since mRNA is not integrated into the genome, electroporation can achieve faster and safer protein expression [201, 202]. In cancer treatment research, researchers have explored electroporation of self-amplifying RNA encoding interleukin-12 (IL-12) into tumor tissues, which significantly prolonged the survival of animal models and enhanced anti-tumor immune responses. When used in combination with PD-1 immune checkpoint inhibition, its therapeutic effect was further improved [203]. Although the strong hydrophilicity, poor membrane permeability and easy degradation of short-chain nucleic acids (such as siRNA, miRNA and their mimics or inhibitors) make their delivery challenging, these molecules can precisely regulate gene expression by electroporation [204, 205]. For example, Huang's team employed micromachining technology to fabricate gold interdigital electrodes on a flexible substrate. By pretreating the skin with a microneedle roller and injecting a conductive liquid, they successfully formed an "*in situ* liquid electrode", which enabled efficient and deep delivery of genes and siRNA at low voltage (Fig. 3(a)) [206]. Building on such advancements in delivery efficiency, studies have demonstrated tangible therapeutic effects: in a mouse tumor model, electroporation-mediated delivery of siRNA targeting VEGF into tumors significantly inhibited tumor growth. This effect was particularly pronounced in models with high VEGF expression, where a single treatment maintained gene silencing for up to 20 days [207]. In recent years, electroporation technology has been further extended to the delivery of the CRISPR-Cas9 gene editing system. By efficiently introducing single guide RNA (sgRNA) [208] and nucleic acid encoding Cas9 protein [209] into target cells, electroporation can achieve precise gene editing *in vivo* [210]. For example, the GONAD (Genome Editing via Fallopian Tube Nucleic Acid Delivery) platform can complete editing in embryos without traditional *in vitro* embryo manipulation [211–214]. Masakazu et al. used electroporation to deliver the CRISPR/Cas9 system to fertilized eggs, achieving gene knock-in mediated by single-stranded oligodeoxynucleotides (ssODN) in mouse

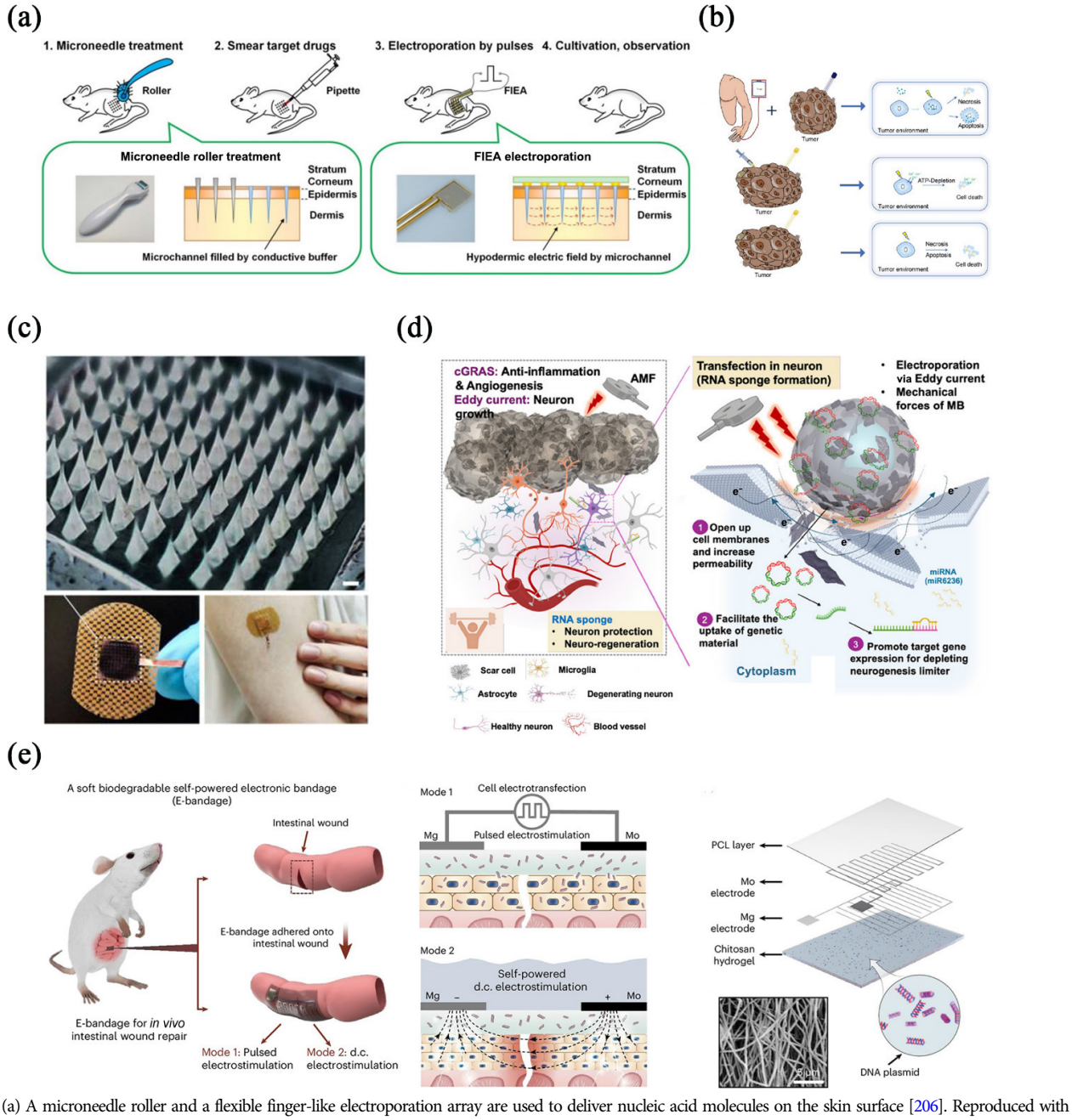


Figure 3 (a) A microneedle roller and a flexible finger-like electroporation array are used to deliver nucleic acid molecules on the skin surface [206]. Reproduced with permission from Ref. [206], © Ivyspring International Publisher 2026. (b) Schematics of electroporation-related tumor therapies: Electrochemotherapy; calcium electroporation; irreversible electroporation [227]. Reproduced with permission from Ref. [227], © Gong, X. et al. Licensee MDPI, Basel, Switzerland 2022. (c) An illustrative application of using a device loaded with OVA plasmid μEPO on the human arm [235]. Reproduced with permission from Ref. [235], © Wang, Y. et al. Published by PNAS 2024. (d) Injecting the miS@cGRAS into the TBI wound enables targeted, on-demand delivery of nucleic acid drugs and electron transfer through mechanical stress signaling pathways from the hydrogel, coupled with AMF stimulation. This process promotes angiogenesis, neurogenesis, and functional recovery during TBI treatment [247]. © Wiley-VCH GmbH 2025. (e) Right: Schematic illustration of the application of the dual-electrostimulation E-bandage onto an intestinal wound molecules on the skin surface. Middle: Low-voltage and continuous d.c. electrostimulation (ES) to the local intestinal epithelial cells enabled by the body fluid-constituted galvanic cell. Left: An exploded view of the layers of the E-bandage [173]. Reproduced with permission from Ref. [173], © Wu, H. et al. under exclusive licence to Springer Nature Limited 2024.

embryos [215]. This method is not only applicable to small animal models such as mice but also shows excellent editing efficiency and accuracy in large animals such as cattle, showing broad prospects in the field of agricultural biotechnology [216]. The latest development of i-GONAD technology has extended *in vivo* gene editing to somatic cells and germ cells, expanding its application possibilities in neuroscience, developmental biology and regenerative medicine [217]. In addition, the electroporation platform combined with microfluidic chips has been applied to the genome editing of human embryonic stem cells, significantly improving the editing accuracy and reducing the risk of off-target effects [217–219].

4.2 Cancer treatment and immunotherapy

Beyond its well-established role in facilitating gene delivery and

genome editing, *in vivo* electroporation has increasingly gained attention as a versatile platform for cancer therapy and immunomodulation. The same principles that enable efficient transfection of therapeutic genes can be leveraged to deliver tumor antigens, immune modulators, and chemotherapeutic agents directly into targeted tissues. This transition from molecular-level interventions to systemic therapeutic strategies underscores the growing potential of electroporation in oncological applications, particularly in enhancing tumor-specific immune responses and improving the efficacy of cancer immunotherapies [17].

IVE has a long and mature application history in enhancing the uptake of small molecule cytotoxic drugs, forming the widely used ECT [220]. In this therapy, doctors inject small molecule chemotherapy drugs with a molecular weight less than 2 kDa such as bleomycin or cisplati locally into or around tumor tissues and apply electric pulses to transiently increase cell membrane permeability, thereby significantly improving the cellular uptake efficiency and bioavailability of drugs [221]. Since the drug effect is mainly concentrated in the local area where the electric pulse is applied, ECT can effectively reduce systemic toxicity and show good therapeutic targeting.

At present, ECT has been widely used in the local treatment of solid tumors, especially in the fields of head and neck tumors, and skin cancer, and has achieved remarkable results. For example, common chemotherapy drugs such as cisplatin and carboplatin have achieved synergistic killing effects in various solid tumor models after being combined with electroporation technology [222–225]. Particularly in head and neck tumors, a standardized protocol is proposed. This involves delivering eight 100 μs pulses at a frequency of 5 kHz, with an applied electric field of 1 kV/cm. Electrode selection is critical and depends on tumor characteristics: finger or plate electrodes are preferred for superficial, smaller tumors, while needle electrodes are employed for deeper, more substantial lesions. Concurrently, a combination of bleomycin and cisplatin is administered intratumorally at a dosage of 1000 IU/m^2 [187]. The turmeric-silver nanoparticle (TurNP) combined with ECT proposed by Mittal et al. significantly enhanced the cytotoxic effect in the treatment of triple-negative breast cancer, providing a new idea for drug delivery strategies . The core advantage of ECT lies in its physical enhancement mechanism: electric pulses can instantly change the cell membrane structure, so that the intracellular concentration of extracellular drugs can be instantly increased by hundreds to thousands of times, thereby significantly enhancing cytotoxicity and improving tumor ablation efficiency [5, 226]. This mechanism not only has therapeutic advantages for tumors resistant to traditional chemotherapy, but also greatly promotes the development of electroporation in a variety of derivative therapies, such as calcium electroporation, gene electro-transfer and IRE (Fig. 3(b)) [227].

In addition to the enhancement of traditional cytotoxic drugs, the potential of electroporation technology in tumor immunotherapy is gradually emerging. In particular, in terms of DNA delivery, electroporation has been shown to effectively induce the activation of immune responses. A clinical study of melanoma patients showed that direct injection of plasmid DNA encoding IL-12 into the tumor and supplemented with electroporation can induce tumor necrosis and a large number of lymphocyte infiltration, and not only the tumor at the treatment site degenerated, but also the distant metastatic lesions showed signs of shrinkage [228]. This study indicates that electroporation delivery can trigger both local anti-tumor effects and systemic immune responses, suggesting its potential to bridge local treatment approaches with systemic immune modulation in cancer therapy. To enhance its effectiveness, researchers are focusing on developing more precise and personalized electroporation parameters and platforms tailored to the specific delivery needs of different tumor types and immune targets. Amidst these efforts, the strategy of combining electroporation with antibody therapy has garnered increasing attention. By improving the efficiency of tumor antigen presentation, electroporation holds promise for enhancing the immune system's ability to recognize and eliminate tumor cells [227]. Although *in vivo* direct protein delivery technology remains exploratory, studies are applying it to cell engineering. For instance, electroporation has been used to introduce Cas9 ribonucleoprotein (RNP) into T cells, generating chimeric antigen receptor T cells (CAR-T) that are subsequently reinfused for precision immunotherapy. This approach not only streamlines CAR-T cell production but also significantly mitigates the risks associated with viral vectors [229–231].

As a multifunctional treatment strategy that combines local efficient delivery and cell killing, ECT is gradually integrating a variety of advanced technologies such as immunotherapy, gene therapy and nano-drug delivery. As a physical enhancement platform, it demonstrates unique comprehensive advantages in improving tumor treatment effects, breaking through drug resistance mechanisms, and achieving systemic anti-tumor immune responses. It is expected to play an increasingly important role in precision cancer treatment in the future.

4.3 Vaccination and infectious diseases

IVE has emerged as a critical platform for nucleic acid vaccine delivery, markedly enhancing cellular uptake and antigen expression, thereby eliciting robust and sustained immune responses. The emergence of electroporation technology has greatly promoted the iteration and upgrading of nucleic acid vaccines and has shown excellent efficacy in the research and application of DNA vaccines and RNA vaccines.

Electroporation significantly potentiates the efficacy of DNA vaccines by establishing broad-spectrum immunity. Studies have demonstrated that IVE can elicit robust and sustainable antibody responses against malaria transmission-blocking antigens in murine models [115, 232]. Furthermore, IVE efficiently delivers multivalent DNA vaccines targeting complex pathogens — such as human immunodeficiency virus type 1 (HIV-1) and Zika virus — thereby activating comprehensive humoral and cellular immune responses [233, 234]. Wang et al. integrate flexible electronics with micro-nano processing. This device features a unique three-dimensional structure of "hydrogel loading-micro-needle-micro-electrode", this device functions like a "band-aid", enabling the safe and rapid transfection of macromolecules into epidermal and dermal cells at low voltages. In ovalbumin (OVA) cancer vaccine model validation demonstrated that this device can effectively activate antigen-specific immune responses, resulting in interferon-gamma$^+$ (IFN-γ^+) cytotoxic T lymphocytes generating a 10-fold dose-saving advantage, and effectively inhibiting tumor growth in rodents, providing a new approach for the DNA vaccine strategy in cancer immunotherapy (Fig. 3(c)) [235]. Such innovations represent a paradigm shift from traditional invasive intramuscular injections to minimally invasive or non-invasive transdermal modalities. The advent of adaptive electroporation platforms has further enhanced

the consistency, reproducibility, and therapeutic efficiency of DNA vaccine delivery through the real-time optimization of electrical pulse parameters [236, 237]. In clinical trials, EP-mediated DNA vaccines have exhibited robust protective immunity against refractory infectious diseases, including Zika [238], Ebola [239], MERS-CoV [240], and HIV [241]. Among these approaches, intradermal electroporation (ID-EP) is particularly distinguished by its superior tolerability and dose-sparing potential [240]. Mechanistically, ID-EP delivers DNA directly into cutaneous antigen-presenting cells (APCs), which subsequently migrate to draining lymph nodes to prime potent cell-mediated and humoral immune responses [128, 242].

In the field of RNA vaccines, electroporation demonstrates significant advantages, unlocking the potential of mRNA vaccines and emerging as a pivotal technology for combating emerging infectious diseases. Since mRNA expresses antigens directly in the cytoplasm without needing to enter the cell nucleus, electroporation markedly enhances its delivery efficiency and the intensity of the elicited immune response. Preclinical studies indicate that electroporation can boost mRNA-derived protein expression by up to 100-fold, achieving immune efficacy comparable to, or even surpassing, high-dose DNA vaccines [243]. These benefits have been successfully converted into strong immune protection in animal models, particularly evident in the development of vaccines against emerging pathogens like COVID-19 and respiratory syncytial virus (RSV) [244]. As the mRNA vaccine platform continues to expand, electroporation has become a key tool for enhancing vaccine efficacy, accelerating development timelines, and enabling more precise immunity.

Electroporation not only significantly enhances the immunogenicity of DNA and RNA vaccines but also expands the application prospects of nucleic acid vaccines in responding to emerging infectious diseases, improving herd immunity coverage and vaccine dose economy. With the continuous optimization of equipment technology and delivery strategies, electroporation is expected to play a more core role in future large-scale vaccine deployment and global public health prevention and control systems.

4.4 Tissue engineering and regeneration

The application of IVE in regenerative medicine has emerged as a transformative approach for delivering therapeutic genes or bioactive molecules to promote tissue repair, regeneration, and cellular reprogramming. One of the most extensively validated applications of IVE is wound healing, where delivery of angiogenic genes such as VEGF enhances vascular density, accelerates tissue regeneration, and improves functional outcomes [245]. For instance, localized pVEGF plasmid electroporation has been shown to accelerate perfusion and collagen deposition in ischemic wounds and skin defects [246]. The application of IVE in the field of tissue repair is continuously expanding, providing innovative ideas for the treatment of various refractory injuries. For traumatic brain injury (TBI), conductive particle scaffolds (cGRAS) have been developed to target miRNA for regulating neural repair. They can alleviate TBI inflammation and glial scar formation, generate electrical stimulation under external alternating magnetic fields (AMF), and promote time electroporation. Combined with mechanical conduction inhibition of miR6263 overexpression, they ultimately achieve inhibition of inflammation in the injured area, improvement of brain function, and recovery of behavior (Fig. 3(d)) [247]. For the problem of intestinal wound healing, self-powered biodegradable electronic bandages function through pulsed electrical stimulation to induce electrical transfection of intestinal epithelial cells to promote the expression of healing factors, and enhance the secretion of these factors through direct current stimulation. The self-powered system composed of magnesium-molybdenum microelectrodes ensures the realization of functions, and *in vitro* and *in vivo* experiments have confirmed that its healing effect is superior to traditional suturing and single electrical stimulation devices (Fig. 3(e)) [173].

IVE also facilitates delivery of anti-inflammatory cytokines or tissue-specific transcription factors, which promote resolution of inflammation and regeneration of damaged tissues. This approach is especially valuable in chronic wounds, where conventional treatments often fail to initiate effective healing responses. In neuroregeneration, IVE has enabled the targeting of retinal ganglion cells, spinal cord neurons, and peripheral nerves, facilitating gene-mediated functional repair and neuronal trans-differentiation. Importantly, in utero and neonatal electroporation protocols have opened new avenues for investigating early brain development, neural circuit formation, and congenital disorders. The basic steps of in utero electroporation in embryos for studying the molecular mechanisms of brain diseases are introduced [86]. These techniques support high-resolution spatiotemporal gene expression, allowing for real-time lineage tracing and disease modeling [248]. Electroporation has also found promising applications in cardiac repair, especially after ischemic injury. By delivering cardioprotective or angiogenic genes such as VEGF or hypoxia-inducible factor 1-alpha (HIF-1α) to myocardial tissue, IVE has been shown to improve perfusion, limit infarct expansion, and restore contractile function [249, 250]. Given the dynamic mechanical environment of the heart, novel electrode systems including flexible arrays and synchronized pulse platforms are being developed to optimize electroporation in cardiac applications. Beyond tissue repair, IVE has proven effective in cellular reprogramming, serving as a non-viral platform for converting somatic cells into induced pluripotent stem cells (iPSCs), or for direct lineage conversion (e.g. fibroblasts to neurons or endothelial cells). The delivery of reprogramming transcription factors via mRNA electroporation provides a safer alternative to viral vectors by minimizing risks of insertional mutagenesis while enabling transient, tunable expression [251]. To achieve delivery precision enhancement, electroporation is now being coupled with biomaterial platforms — including injectable hydrogels and scaffolds. These composite delivery systems confine genetic payloads to target sites, allow controlled release of co-factors, and improve cellular retention at injury locations. Such integrated approaches are actively explored acrosss orthopedic, dermatological, and cardiovascular regenerative therapies [252].

IVE technology demonstrates a wide range of therapeutic versatility, covering multiple key medical fields such as gene defect repair, tumor immunotherapy, vaccine development, and tissue regeneration. With the spatial controllability of molecular delivery and the ability to adapt to multiple vectors in non-viral ways, IVE has built a highly flexible and safe precision medicine platform. With the continuous accumulation of clinical applications and the continuous optimization of equipment engineering, IVE is expected to be deeply integrated with personalized treatment strategies, further expanding its application boundaries in different medical disciplines, and laying a solid foundation for building a safer, more efficient and scalable intervention system.

5 Clinical translation and current trials

With advancing insights into cellular electrophysiological, IVE technology has evolved from foundational experimental research to a clinically promising medical strategy. During preclinical development, researchers systematically evaluated the interactions between different electrical pulse parameters, tissue types and delivered molecules, and established preliminary safety and efficacy protocols. Successful validation across diverse animal models further promoted the potential application of IVE in the treatment of genetic diseases, cancer and immune-related diseases. Subsequent early-phase clinical trials confirmed the advantages of IVE in targeting, delivery efficiency and patient tolerance, laying a critical foundation for larger-scale clinical studies [15]. The clinical translation pathway for IVE encompasses preclinical validation, early safety assessment, and late-phase multicenter trials. Each stage drives concurrent optimization of technical parameters, refinement of treatment protocols, and enhanced safety profiles. Table 3 summarizes current IVE-delivered therapeutic molecules, target disease areas, and corresponding clinical development stages.

Over the past two decades, the clinical translation of IVE has made significant progress. A large amount of clinical data supports its application prospects in gene therapy, oncology, vaccination and immune regulation, demonstrating favorable safety, feasibility and therapeutic potential. This section will outline the current key clinical outcomes to date, summarize the progress of *in vivo* electroporation, and analyze the critical challenges for widespread translational application.

5.1 Electroporation-enhanced vaccination

Application of IVE in the field of vaccination has become one of the most cutting-edge and transformation-potential clinical directions. Gene electrotransfer (GET), as an important derivative form of electroporation technology, is the most mature and widely used

Table 3 List of drugs delivered by *in vivo* electroporation, types of disease treated, and clinical advances

Types of drugs delivered	Treatment cycles	Advantages compared with traditional treatments	Types of diseases treated	Clinical trial stages	Ref.
DNA vaccines	Repeated cycles (multi-dose)	High transfection efficiency with reduced toxicity versus viral vectors	Cancer, infectious diseases	Phase I/II	[253]
	Single injection with booster option	Significantly enhanced immune response compared with conventional injection	HPV-associated tumors	Preclinical/Phase I	[81]
	Single administration with booster options	Significantly improved immunogenicity compared with conventional injections	Infectious diseases and cancer	Phase I/II trials	[253]
	3–5 cycles	Enhanced gene delivery precision, reduced toxicity	Cancer (melanoma, cervical)	Phase I/II	[188]
	Multiple cycles	Non-viral delivery, increased efficacy	Cancers, genetic disorders	Phase I/II	[254]
	Single/multiple	High delivery rate with spatial targeting	Muscle disorders, gene therapy	Preclinical/translational	[255]
	2–4 doses	Boosted immune response, low systemic exposure	Cancer, infectious diseases	Phase I/II	[115]
	Multidose	Minimally invasive, high safety	Cervical cancer, melanoma	Phase I	[256]
	3 cycles	Stronger T-cell response	HIV	Phase I	[257]
Chemotherapeutic agents & IRE	Multiple cycles	Enhanced local drug uptake and minimized systemic side effects	Solid tumors in human & veterinary oncology	Clinical application (Phase I/II)	[258]
	Multiple treatment cycles	Enhanced local uptake and reduced systemic toxicity versus systemic chemotherapy	Solid tumors in human and veterinary oncology	Phase I/II	[258]
	3 sessions	Enhanced tissue ablation, precision control	Liver, pancreas, kidneys pancreatic cancer	Phase I	[259]
	1–2 sessions	Real-time monitoring & efficacy control	Topical cancers	Early clinical	[260]
	1–3	Nonthermal destruction with margin preservation	Liver tumors	Clinical application	[261]
	Varied	Nonthermal ablation, tissue specificity	Pancreas, liver, nerves	Exploratory	[262]
Peptides & small molecules	Single/multiple	Improved bioavailability, low invasiveness	Skin disorders	Preclinical	[263]
	1–2 sessions	High efficacy, patient comfort	Skin, cosmetic	Preclinical	[264]
	Variable	Less systemic side-effects	Dermatological diseases	Translational	[265]
	Variable	High transfection rates, low risk	Multiple diseases	Various stages	[266]
	Flexible	Magnetically guided, efficient penetration	Tumors	Phase I	[267]
New carriers, RNA and comprehensive delivery	2–4 cycles	Non-invasive targeting	Liver, heart	Translational	[268]
	Single/multiple	Cell-specific high-load delivery	Various cell types	Translational	[269]
	Single or booster treatments	Precise dose control high cell viability versus bulk electroporation	gene therapy	Preclinical	[192]
	Multicycle	High dose concentration in tumor	Various cancers	Phase I/II	[256]

strategy for DNA and mRNA vaccine delivery. In preclinical studies, a large amount of evidence has shown that GET can stimulate high levels of humoral and cellular immune responses, providing a solid foundation for its entry into the human trial stage [270, 271]. With the safety of non-viral delivery and efficient cell uptake ability, GET is actively promoting the clinical development and transformation of a series of infectious disease vaccines and therapeutic tumor vaccines. In clinical practice, ID-EP combined with DNA vaccines has been proven to be a safe and effective vaccine delivery mode. Taking the SARS-CoV-2 spike protein DNA vaccine INO-4800 developed during the COVID-19 pandemic as an example, the results of its Phase I clinical trial showed that intradermal injection combined with electroporation delivery can induce up to 95%–100% serum conversion rate and T cell response rate, showing excellent immunogenicity [272]. In addition, IVE has also made positive progress in the research of vaccines for various other infectious diseases. For example, in a Phase I clinical trial for Zika virus, the DNA vaccine delivered by electroporation not only triggered a strong humoral and cellular immune response but also showed good safety and a very low incidence of adverse events [272]. Similarly, a Phase I open-label trial of a DNA vaccine for middle east respiratory syndrome coronavirus (MERS-CoV) showed dose-dependent immunogenicity [273]. These results not only verify the effectiveness of the vaccine itself but also emphasize the clinical feasibility of the electroporation delivery system. The CELLECTRA® electroporation device used in these trials is particularly critical. The system features programmable constant current pulse output, which can improve the efficiency of gene delivery while minimizing patient discomfort, thereby improving clinical acceptance and compliance [274]. Beyond its role in infectious disease prevention, IVE also holds great promise in cancer immunotherapy, particularly as an effective delivery platform for therapeutic tumor vaccines. Therapeutic DNA vaccines, exemplified by VGX-3100, offer a novel approach for the treatment of high-grade cervical intraepithelial neoplasia (CIN2/3) by targeting the key human papillomavirus (HPV) oncoproteins E6 and E7. Administered via intramuscular injection combined with IVE, this delivery method significantly enhances cellular uptake of the plasmid DNA, thereby stimulating a specific $CD8^+$ T-cell immune response against the E6/E7 antigens. Clinical trials have confirmed that this strategy effectively leads to histological regression of lesions and clearance of the HPV infection [275], thus providing a promising non-surgical treatment option for patients with CIN2/3 [256].

Currently, clinical-grade electroporation platforms represented by the CELLECTRA® series of devices developed by Inovio have been widely used in clinical research on DNA vaccines, covering multiple indications such as AIDS, hepatitis C, Zika virus, and influenza. The CELLECTRA®-3P dermal electroporation device (Fig. 4(a)) [106] has been used in multiple human studies to enhance the immune response to influenza vaccines and has been shown to have excellent clinical applicability, safety, and platform compatibility [272, 273, 275, 276].

5.2 Oncology applications: ECT and IRE

After years of preclinical studies in mouse and primate models, along with ongoing clinical exploration, the efficacy of ECT in treating various tumors has been widely validated. Its standardized procedural workflow has been established and is widely applied in the treatment of skin metastases-particularly melanoma-as well as head and neck tumors and other malignancies. Its therapeutic mechanism includes inducing tumor cell death, releasing tumor-associated antigens, and activating the body's adaptive immune response, further forming anti-tumor immune memory [187, 277, 278].

A large number of clinical case reports have shown that for melanoma skin metastases that are resistant to conventional treatment and cannot be surgically removed, the use of bleomycin combined with ECT can achieve significant local lesion control and relieve patient-related symptoms [220]. Based on these reliable clinical evidence, international treatment guidelines have officially included ECT in the standard local treatment paradigm for specific types of skin cancer (such as malignant melanoma and non-melanoma skin cancer) [279]. With the development of medical imaging-guided technology, the scope of ECT has been successfully expanded from superficial tumors to deep solid tumors [279]. Notably, ECT demonstrates unique advantages for treating liver malignancies, particularly tumors adjacent to major blood vessels where thermal ablation is contraindicated due to the "heat sink effect". Image-guided ECT has been validated as a safe and effective alternative therapy [280, 281]. A study on colorectal liver metastases showed an encouraging result that after ECT treatment, patients achieved an 85% complete remission rate and a 15% partial remission rate in imaging assessment [282].

To further improve efficacy and optimize patient experience, ECT technology is still evolving. Current research is focusing on new high-frequency electrical pulse strategies to improve electroporation efficiency, reduce pain, and simplify the operation process. For example, high-frequency chemotherapy regimens that can couple multiple electrodes (Fig. 4(b)–4(d)) are becoming an important research direction [283]. These innovative explorations show that ECT, as a core electroporation technology, will play an increasingly critical role in the field of precision tumor treatment in the future. Meanwhile, calcium electroporation (CaEP), as a cutting-edge intervention strategy for solid tumors, has entered the early clinical evaluation stage. This technology promotes the influx of calcium ions through electric pulses, destroys the ion homeostasis in tumor cells, thereby inducing cell apoptosis and achieving selective killing of tumors [284]. In many clinical studies, ECT has also shown significant effects in the treatment of head and neck tumors, not only enhancing tumor regression response, but also helping to prolong patient survival [285]. In skin and soft tissue tumors (such as melanoma), ECT has been proven to effectively improve local control rate and patient overall response rate. These results fully demonstrate that electroporation-based treatment is not only reliable in terms of efficacy but can also serve as an important supplement and enhancement to existing treatment methods.

As a unique non-thermal ablation method, IRE has been established as an important tissue ablation technique in clinical practice, especially for complex cases where the risks of traditional thermal ablation are high [286]. Its core mechanism relies on applying high-voltage, microsecond-level short-term electrical pulses to form nanoscale permanent pores in the cell membrane. This process destroys the integrity of the cell membrane, thereby inducing cell apoptosis rather than necrosis, significantly reducing inflammatory responses and tissue damage [43]. The defining advantage of IRE lies in its non-thermal mechanism: it achieves effective target tissue ablation while maximally preserving surrounding extracellular matrix, vasculature, nerves and other

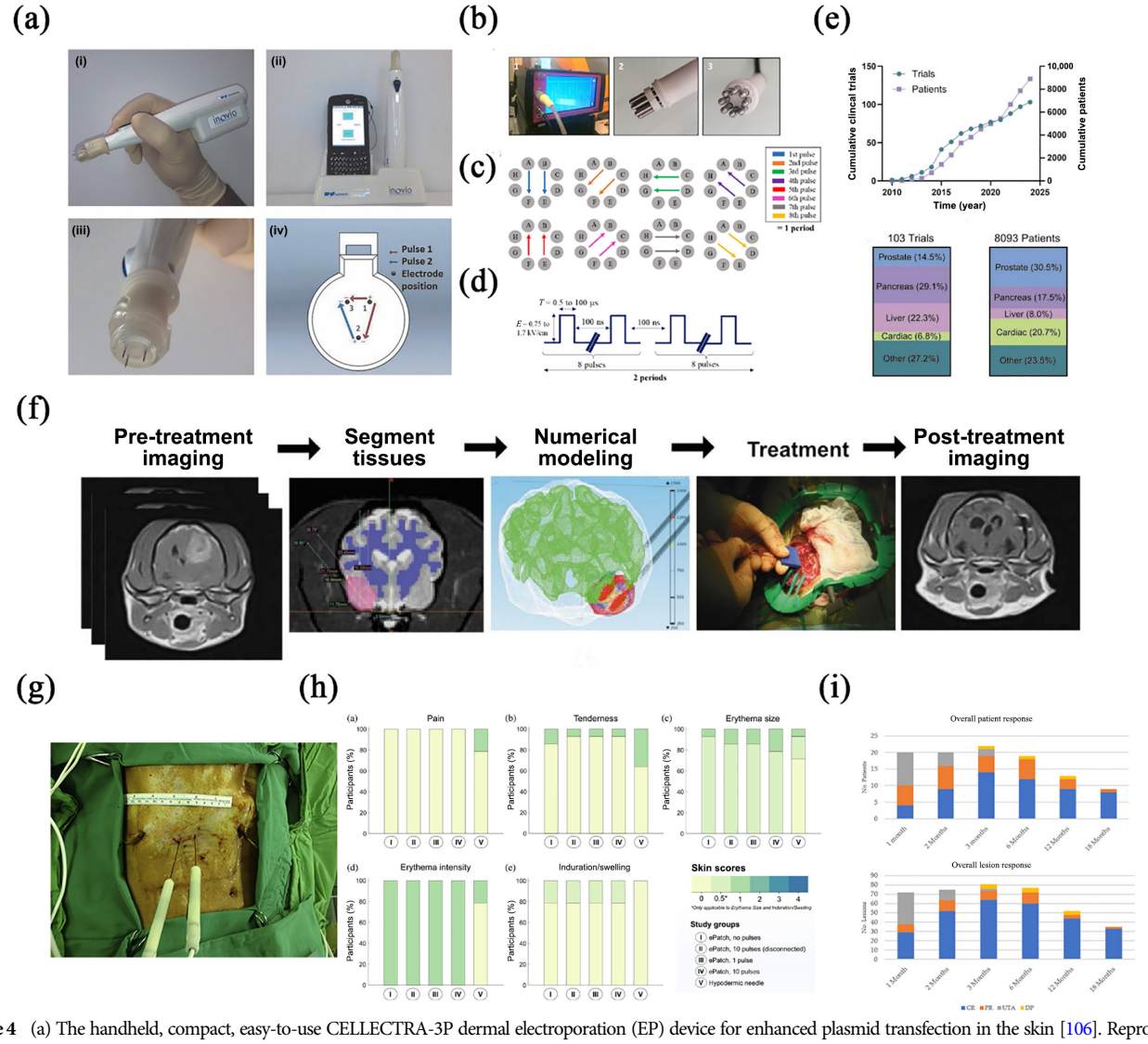


Figure 4 (a) The handheld, compact, easy-to-use CELLECTRA-3P dermal electroporation (EP) device for enhanced plasmid transfection in the skin [106]. Reproduced with permission from Ref. [106], © Mary Ann Liebert, Inc. 2005. (b) High frequency ECT protocols. (c) ECT device was coupled with multipolar electrodes. (d) Example of protocol used with H-FECT procedure [283]. Reproduced with permission from Ref. [283], © Elsevier B.V. All rights are reserved, including those for text and data mining, AI training, and similar technologies 2025. (e) Cumulative registered patient and trial numbers for IRE and PFA on ClinicalTrials.gov.Breakdown of trials and patient populations by tissue type [290]. (f) Pulsed field ablation treatment planning pipeline. Images of the region of interest are taken through CT, MRI, US, or other modalities [290]. Reproduced with permission from Ref. [290], © Edward J Jacobs, Boris Rubinsky, Rafael V Davalos, published by Association of Radiology and Oncology 2025. (g) Percutaneous application of IRE guided using ultrasound or CT [291]. Reproduced with permission from Ref. [291], © Elsevier Taiwan LLC and the Chinese Taipei Society of Ultrasound in Medicine 2017. (h) Skin tolerability immediately after application of ePatch and hypodermic needle control in human subjects [296]. Reproduced with permission from Ref. [296], © Lu, C. Y., et al. Bioengineering & Translational Medicine published by Wiley Periodicals LLC on behalf of American Institute of Chemical Engineers 2024. (i) Overall patient response and lesion response for all histological subtypes at each follow-up time point [305]. Reproduced with permission from Ref. [305], © Lyons, P., et al. Licensee MDPI, Basel, Switzerland 2023.

critical. This unique capability confers irreplaceable clinical value for tumors adjacent to vital anatomy — such as peri-prostatic zones [287] and pancreatic portal vein regions [288] — where conventional thermal ablation techniques are inapplicable.

The clinical indications for IRE continue to expand. Beyond oncology, IRE is being evaluated in cardiac electrophysiology as a non-thermal alternative for atrial fibrillation ablation technology, demonstrating favorable safety and tissue selectivity in clinical studies [289]. Since IRE was put into clinical use in 2010, more than 100 clinical trials have been registered worldwide. The experiments and patients in the IRE clinical trials have been classified according to the type of tissue (Fig. 4(e)) [290]. After obtaining the images of the target areas through imaging methods such as CT, MRI, and ultrasound, tissue segmentation needs to be performed first to define different regions. Then, numerical modeling is used to achieve the maximum coverage of the target tissue and the precise generation of the key electric field, while avoiding damage to adjacent structures (Fig. 4(f)) [290]. Technically, IRE procedures utilize electrode arrays to generate controlled electric fields around the tumor. Ablation zone dimensions can be predicted based on electrode spacing configurations, while percutaneous IRE under ultrasound/CT guidance enhances procedural reproducibility (Fig. 4(g)) [291].

Notably, electrical stimulation therapies now extend into emerging areas such as regenerative medicine and neurodegenerative diseases treatment. Emerging evidence indicates

IRE modulates microglial functional activity [292], suggesting novel therapeutic pathways for neurological disorders. As electrical stimulation devices and protocols increasingly obtain regulatory approvals, IRE is expected to become a cornerstone of precision medicine, advancing minimally invasive interventions through unparalleled targeting accuracy and tissue preservation capabilities.

5.3 Clinician training and patient acceptance

The effective clinical translation of IVE technology depends to a considerable extent on two interrelated human factors: clinician expertise and patient acceptance. While considerable progress has been made in device development and biophysical optimization, the actual clinical utility of IVE hinges on the operator's proficiency and the patient's willingness to participate in these therapies.

Clinicians must undergo rigorous, specialized training to ensure safe and effective administration of electroporation-based interventions. This includes mastery of equipment calibration, precise electrode placement tailored to anatomical structures, understanding tissue conductivity, and continuous real-time monitoring of physiological responses during pulse delivery [293]. These procedures, often performed under time-sensitive conditions or in combination with chemotherapeutic agents, leave little margin for error. As such, institutionalizing structured training curricula and certification protocols across clinical centers is essential to standardize practices and minimize inter-operator variability [294, 295]. Moreover, standard operating procedures (SOPs) should be harmonized globally to facilitate comparative studies, regulatory approval, and quality assurance in multicenter trials.

Simultaneously, patient acceptance represents a crucial yet often underestimated factor in IVE adoption. Patients may express concern about the novel nature of electroporation, including fears of pain, electrical shocks, and the need for multiple treatment sessions. These perceptions can affect enrollment in clinical trials and long-term adherence in therapeutic regimens. Some researchers conducted subjective evaluations of the tolerance of human skin, as well as the pain and tenderness conditions, after applying the ePatch to the subjects and after treatment through subcutaneous injection needles. These evaluations were conducted 10 min after the skin treatment. Meanwhile, clinical researchers assessed the size, intensity of the erythema, and the presence of hard nodules/edema through visual inspection, and used a 0–4 point rating scale for the assessment (Fig. 4(h)) [296]. Evidence indicates that transparent patient education, combined with empathetic communication about the benefits and risks, plays a pivotal role in reducing anxiety and improving compliance [297, 298]. When IVE procedures are described as minimally invasive and well-tolerated, patient satisfaction and acceptance levels tend to improve, which in turn positively impact treatment outcomes [19, 237].

Ultimately, bridging the gap between biomedical engineering and bedside practice requires a holistic implementation strategy. By investing in clinician training infrastructures and addressing the psychosocial dynamics of patient engagement, healthcare systems can build a robust foundation for the widespread and ethical use of IVE in clinical settings. These dual pillars — standardized professional competence and informed patient participation — are indispensable for the sustainable integration of electroporation into modern therapeutic paradigms.

5.4 Challenges in clinical translation

Although IVE has shown significant promise in basic research and early clinical trials, its widespread clinical translation is still limited by many factors. These challenges cover multiple dimensions, including technical implementation, understanding of biological mechanisms, clinical operational procedures, electroporation efficiency and reproducibility, safety and regulatory approval pathways.

From a mechanistic perspective, the biophysical and cellular response mechanisms of electroporation have not yet been fully elucidated, and there are still limitations in understanding the biological mechanisms. From membrane pore formation to cellular response to tissue remodeling and immune response, there are still knowledge gaps in the entire biological causal chain [299]. Uncertainty at the mechanistic level makes it difficult to predict individualized treatment, limits the fine optimization of parameters, and hinders its in-depth integration into the precision medicine system.

The heterogeneity of biological tissues is one of the main technical obstacles facing electroporation, facing challenges in electric field control and delivery accuracy [300, 301]. Human tissues are composed of multiple cell types, complex extracellular matrices, and dense vascular networks, resulting in significant differences in electrical properties such as conductivity and impedance in different regions [39, 302, 303]. The heterogeneity of such physiological structures seriously affects the possibility of achieving uniform and controllable distribution of the electric field in the target tissue, especially in tumor tissues with irregular morphology and complex microenvironment, where insufficient electric field coverage is likely to occur, resulting in incomplete ablation and the risk of tumor recurrence during IRE treatment [304]. In terms of clinical treatment outcomes, researchers conducted a long-term follow-up investigation on the treatment of patients with malignant skin tumors. During the three-month follow-up period, the patients' conditions were evaluated at four levels: complete remission, partial remission, deterioration of the condition, and withdrawal/failure of follow-up. The study showed that the overall remission rate of the 22 patients who continued to participate in the research was 86%; the analysis of 81 lesions revealed a total remission rate of 91% (Fig. 4(i)) [305]. In the context of gene or drug delivery, the transfection efficiency of IVE is still lower than that of viral vectors in many situations, and multiple treatments are usually required to achieve the desired therapeutic effect. Current research is exploring real-time monitoring technologies such as impedance spectroscopy and MRI guidance, as well as high-precision computational models for electric field distribution prediction and personalized parameter control [306, 307], but these methods are still in the early stages of development and have not yet been widely used in clinical practice.

Another major challenge in IVE is performance variability, manifesting as inconsistent transfection efficiency and poor repeatability. Transfection efficacy exhibits significant tissue dependent heterogeneity influenced by electrode geometry, pulse parameters, molecular formation and administration timing [308]. The high interdependence of these variables amplifies sensitivity to minor operational deviations, causing substantial outcome variations across studies and institutions. Furthermore, the absence of standardized pulse parameters and device calibration protocols across institutions further complicates cross-study comparisons and limits reproducibility in multicenter trials [195, 309]. The high interdependence of these variables amplifies sensitivity to minor operational deviations, causing substantial outcome variations

across studies and institutions. Although emerging real-time monitoring systems such as impedance tracking and electroacoustic imaging show promise for adaptive control, their clinical translation remains limited by standardization barriers and implementation challenges [299].

In addition, the physicochemical properties of the injected biomolecules (such as viscosity, concentration, buffer system and stability) will also significantly affect the diffusion of molecules in tissues, electrophoretic transport efficiency and cellular uptake rate [310]. Without a unified formulation standard and operating procedure, it will be difficult to ensure reproducibility between different studies. Therefore, it is necessary to establish a complete standardized operating system covering electrical parameters, tissue pretreatment (hydration status), anesthesia management, postoperative recovery and immune monitoring. Multi-center validation using unified SOPs and interoperable equipment will be crucial to improving reproducibility and promoting clinical implementation.

In clinical practice, the involuntary muscle contractions and procedural discomfort caused by IVE increase the demands on anesthesia management. Additionally, ensuring the safety of high-intensity electric fields near critical organs, such as the heart, remains an important consideration in both device design and operating protocols [311, 312]. Safety has consistently been a primary concern as IVE moves forward routine clinical use. Although considered a non-thermal technique, IVE can still generate localized heating under conditions of high voltage, prolonged pulse duration, or high frequency, potentially leading to tissue damage or altered drug kinetics [313, 314]. Moreover, pain and muscle rigidity from electrical stimulation can significantly impact patient comfort and compliance, particularly in deep tissue and high-dose treatments [315]. Patient-specific factors also play a role in treatment outcomes. Variations in pain sensitivity, tolerance to electrical stimuli, and tissue impedance can influence both effectiveness and acceptance, especially in repeated dosing regimens [316, 317]. Strategies such as general anesthesia, neuromuscular blockade or the use of high-frequency biphasic pulses have been investigated to reduce nerve stimulation while maintaining high transfection efficiency [312]. The local inflammatory response following electroporation — characterized by edema, cytokine release and immune cell infiltration — are another factor to consider. While such responses may enhance immunogenicity in cancer therapy or vaccination, they can hinder tissue regeneration or gene repair, thereby reducing the therapeutic effect [318]. To address this, some studies have explored co-delivering anti-inflammatory or immunomodulatory agents alongside therapeutic payloads to achieve a balance between efficacy and safety [319]. Notably, long-term safety data for IVE remain limited, particularly for repeated or chronic treatment. Potential risks such as fibrosis, chronic pain, peripheral nerve damage and immune sensitization require further investigation through systemic toxicology, biodistribution and extended clinical follow-up [320]. In the future, integrating real-time impedance and temperature monitoring with personalized energy control algorithms may help maintain treatment within a safe therapeutic window and support the long-term clinical adoption of IVE.

Since IVE technology has the dual attributes of tissue ablation and delivery platform in clinical applications, its clinical regulatory approval path is highly complex. When used as a stand-alone ablation device (such as pulsed field ablation, PFA or IRE), its approval path is relatively clear and can be carried out according to traditional medical device processes. When used as a drug or gene delivery platform, IVE constitutes a combined therapeutic system consisting of a device and an active ingredient, and is subject to a more complex joint approval mechanism [297]. This requires not only separate evaluations of the safety and efficacy of the device and drug, but also systematic verification of their combined effects. In addition, during the industrialization stage, system consistency verification, production standardization, and quality control also pose higher challenges.

In summary, although IVE offers a promising non-viral platform for therapeutic delivery, its successful clinical translation will require addressing key technical, biological, and procedural challenges. Standardized protocols to ensure reproducibility, strategies to manage tissue- and patient-specific variability, and safety enhancements through optimized pulse parameters and supportive measures are all essential. Ongoing advancements in pulse engineering, electrode design, and personalized treatment planning will be critical for improving efficiency, tolerability, and outcome predictability. With sustained interdisciplinary collaboration, IVE has the potential to evolve into a versatile and reliable therapeutic modality applicable to a wide range of clinical indications.

5.5 Outlook for IVE in clinical translation

Although notable clinical progress has been achieved, particularly in the fields of therapeutic DNA vaccination and oncology, the full potential of IVE has yet to be realized. Persistent challenges, including technical complexity, inconsistent protocols, and fragmented regulatory frameworks, continue to limit its widespread adoption. The variations in device design, pulse parameters, and dosing strategies require standardization to ensure reproducibility across institutions and patient populations [297, 321]. Encouragingly, next-generation IVE platforms are emerging with simplified interfaces, real-time tissue feedback systems, and adaptive pulsing algorithms, making them more suitable for point-of-care applications [46].

The integration of advanced computational modeling and AI into treatment planning marks a transformative direction. Patient-specific electric field simulations can guide electrode placement and pulse settings, maximize transfection efficiency while minimizing tissue damage [322, 323]. AI-driven platforms can further automate protocol selection based on tumor histology, location, and biomarker expression profiles, paving the way for precision electroporation-based therapies [324].

Ultimately, overcoming current translational barriers will require sustained collaboration among clinicians, biomedical engineers, and industry partners. Such partnerships will accelerate the standardization, automation, and personalization of IVE technologies, enabling them to reach their full therapeutic potential. As technical capabilities and regulatory infrastructures advance, IVE is well-positioned to become a core modality in precision medicine, supporting patient-specific interventions across oncology, vaccinology, and other emerging clinical fields.

6 Future perspectives and innovations

Looking ahead, the clinical prospects of *in vivo* IVE technology are expanding rapidly alongside advances in precision medicine and intelligent technologies. Current research is progressing in several critical pathways to develop more efficient, safer, and broadly applicable therapeutic strategies.

Personalized electroporation approaches have emerged as a frontier research focus. To address individual variability, researchers now employ preoperative imaging such as MRI/CT and personalized modeling to simulate electric field distribution, and dynamically optimize pulse parameters, electrode placement, and treatment duration to improve delivery efficiency and treatment consistency [325]. Systems like PIRET platform and AI-guided electric field estimator have achieved initial success in automating personalized parameter optimization [326–328], transitioning IVE from empirical procedures to mechanism-informed precision treatment [329].

The integration of AI and automated systems is profoundly changing the operation mode and decision-making process of IVE. Machine learning algorithms can perform big data analysis on patient anatomical characteristics, tissue electrical parameters, and treatment results to assist in optimizing pulse parameters and device configuration [328]. The new generation of real-time adaptive systems has embedded AI models into the device to achieve closed-loop regulation based on tissue impedance, local temperature or gene expression [330]. These innovations not only improve the treatment consistency and safety, but also facilitate intraoperative plan adjustments while reducing operator burden [331].

In terms of device development, the IVE platform is evolving towards minimally invasive, wearable and patient-friendly directions. Novel devices such as microneedle arrays and flexible skin patch electrodes significantly reduce skin trauma and procedural discomfort while maintaining delivery efficiency, proving particularly valuable for intradermal immunization and gene vaccination applications [332, 333]. Some devices also integrate impedance or temperature sensors to enable automatic adjustment of pulse parameters, demonstrating potential for home-based treatment and self-management [334]. The application of new materials such as stretchable electronics, biodegradable electrodes and conductive polymers further aligns IVE with the precision medicine's evolving requirements for personalization and sustainable development.

Furthermore, IVE is evolving from a single delivery method to a part of multimodal treatment platform. Collaborative approaches with nanocarriers (nanoparticles, liposomes, hydrogels or cell-penetrating peptides) enhance molecular stability, targeting and cellular uptake efficiency [335–337]. For instance, electroporation can promote the cytoplasmic release of liposome drugs or allow nanocomplexes to escape from endosomes, thereby significantly improving transfection efficiency and reducing tissue damage [338]. Further combination strategies such as the co-delivery of mRNA, siRNA, immune adjuvants and other active ingredients are valuable for cancer immunotherapy, gene correction and tissue regeneration [339], as well as integration with physical stimulation modalities such as ultrasound, magnetic transmission and photothermal response to create spatiotemporally controlled intelligent delivery platforms [161, 340].

In summary, IVE continues to advance toward personalized, intelligent, minimally invasive, and multimodal integration. Innovations in adaptive control systems, flexible devices, and efficient delivery platforms progressively enhance therapeutic precision and clinical utility. As these technologies mature and transition to practical implementation, IVE is poised to become a key non-viral delivery modality within the precision medicine, spanning gene therapy, immunotherapy, and regenerative medicine, propelling patient-directed treatments to new levels.

Data availability

All data needed to support the conclusions in the paper are presented in the manuscript. Additional data related to this paper may be requested from the corresponding author upon request.

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Declaration of competing interest

All the contributing authors report no conflict of interests in this work.

Author contribution statement

Y. W. was responsible for writing the entire manuscript. X. Y. R. compiled and summarized the relevant literature. C. B. L. conceived the logical framework of this review. Y. H. C. and Y. C. contributed to the editing of the manuscript and provided financial support. All the authors have approved the final manuscript.

Informed consent

Not applicable.

Ethics statement

Not applicable.

Use of AI statement

None.

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