Mathematical modeling of the thermal effects of irreversible electroporation for in vitro, in vivo, and clinical use: a systematic review

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ABSTRACT

Introduction: Irreversible electroporation (IRE) is a relatively new ablation method for the treatment of unresectable cancers. Although the main mechanism of IRE is electric permeabilization of cell membranes, the question is to what extent thermal effects of IRE contribute to tissue ablation.

Aim: This systematic review reviews the mathematical models used to numerically simulate the heat-generating effects of IRE, and uses the obtained data to assess the degree of mild-hyperthermic (temperatures between 40 °C and 50 °C) and thermally ablative (TA) effects (temperatures exceeding 50 °C) caused by IRE within the IRE-treated region (IRE-TR).

Methods: A systematic search was performed in medical and technical databases for original studies reporting on numerical simulations of IRE. Data on used equations, study design, tissue models, maximum temperature increase, and surface areas of IRE-TR, mild-hyperthermic, and ablative temperatures were extracted.

Results: Several identified models, including Laplace equation for calculation of electric field distribution, Pennes Bioheat Equation for heat transfer, and Arrhenius model for thermal damage, were applied on various electrode and tissue models. Median duration of combined mild-hyperthermic and TA effects is 20% of the treatment time. Based on the included studies, mild-hyperthermic temperatures occurred in 30% and temperatures ≥50 °C in 5% of the IRE-TR.

Conclusions: Simulation results in this review show that significant mild-hyperthermic effects occur in a large part of the IRE-TR, and direct thermal ablation in comparatively small regions. Future studies should aim to optimize clinical IRE protocols, maintaining a maximum irreversible permeabilized region with minimal TA effects.

1. Introduction

Irreversible electroporation (IRE) is a relatively new ablation modality for the treatment of unresectable cancers [1]. Contrary to contemporary ablation techniques such as radiofrequency ablation (RFA) and microwave ablation (MWA), IRE is proposed as a nonthermal ablation technique [2]. Instead, IRE uses ultra-short, high-voltage pulses between electrodes to induce an altered membrane potential, which leads to the irreversible permeabilization of the membranes of tumor cells through the creation of nanopores [3]. These nanopores allow for increased transmembrane transport, which causes a disturbance in the cell’s homeostasis and consequent cell death through accidental and regulated cell death [4]. Since the proposed mechanism of IRE is nonthermal, the technique is supposedly less prone to a heat-sink effect, which is known to hamper the efficacy of RFA and MWA when performing ablations in the presence of large blood vessels [5]. Another possible advantage of IRE over thermal modalities includes the preservation of vital structures (e.g. blood vessels, bile ducts) traversing the ablation zones, since fibrous and collagen structures are typically not affected by IRE [6]. Therefore, the application of IRE is widely investigated for several indications, such as renal and prostate tumors [7,8], pancreatic cancer [9–12], and perihilar cholangiocarcinoma [10].

However, contrary to the current assumption that IRE is a nonthermal ablation modality, several studies do report on
the presence of thermal ablative (TA) temperatures (T ≥ 50 °C) within the IRE ablation zones during treatment, with histological signs of thermal damage [13–16]. Moreover, it is presently unknown whether the reported hyperthermic effects of IRE, caused by slightly less elevated (mild-hyperthermic (MH)) temperatures 40 ≤ T [°C] < 50 [3,17–19], have a beneficial impact on the irreversible permeabilization effect (IPE; we chose IPE instead of IRE effect to distinguish between only the permeabilization effect, and the permeabilization and the thermal effects jointly produced by IRE), or could be seen as an unwanted side-effect. Potentially, temperature increase during IRE ablation might be needed to enhance the IPE and therefore contribute to a successful ablation [20]. On the other hand, IRE could cause thermal damage to vital structures if applied in the vicinity of thermally vulnerable structures. This leaves the true mechanism of work of IRE to be contentious.

Investigating the degree of mild-hyperthermic and thermally ablative effects in the IRE-treated region (IRE-TR) could provide more insight into the IRE process. Determination of these ratios in vivo or in clinical applications is challenging, since a limited amount of IRE settings and parameters can be explored. Simulations using mathematical models could therefore be very useful. In such simulations, the IRE settings and model parameters can be varied extensively, resulting in improvement of our understanding of the IRE mechanism.

Therefore, the aim of this systematic review is to summarize and review the mathematical models used to numerically simulate the heat-generating effect of IRE, and to assess the degree of mild-hyperthermic and thermally ablative effects of IRE in relation to the IRE-TR, as predicted by the simulation results provided by the studies that were included in this review. Accordingly, we investigated whether the analytical/numerical model calculations reported for IRE in the literature could be used to answer the following research questions:

- Does IRE produce a thermal elevation that results in mild-hyperthermic effects?
- Does IRE produce a thermal elevation that results in thermally ablative effects?
- What are the relative sizes expressed in percentages of the regions with the mild-hyperthermic and thermally ablative effects in the IRE-TR?

2. Methods

This systematic review was performed according to the Preferred Reporting Items for Systematic Reviews and Meta (PRISMA)-analyses guidelines [21,22]. In this article, the main data and equations were extracted, analyzed, and reviewed. Additional description of data or equations was added to Supplementary Appendices 1–6:

- Supplementary Appendix 1: Full description of the search strategies.
- Supplementary Appendix 2: Definitions of abbreviations, symbols, and extracted parameters.
- Supplementary Appendix 3: Extended mathematical description of IRE.
- Supplementary Appendix 4: Additional clarification of definitions.
- Supplementary Appendix 5: Extended mathematical description applied to methods of analysis.
- Supplementary Appendix 6: Extended summary of study characteristics and results from included studies.

2.1. Study selection

The medical and technical databases and search engines (Cochrane Library, Embase, IEEE Xplore Digital Library, PubMed, ScienceDirect, Scopus, and Web of Science) were searched for journal articles from inception up to and including 8 February 2019 using search terms related to ‘electroporation’, ‘mathematical models’, and ‘temperature’. A full description of the search strategy can be found in Supplementary Appendix 1. After removing the duplicates and excluding studies written in any other language than the English language, we searched for the terms ‘irreversible electroporation’ OR ‘ablation’ using EndNote X9, restricting the search terms to ‘Any Field’. Subsequently, the titles, the abstracts, and the full-text were screened for eligibility based on predefined inclusion and exclusion criteria that can be found in Sub section 2.2. Any disagreement was resolved by discussion between the review authors (PA) and (EvV). The flow diagram of the study selection is shown in Figure 1.

2.2. Eligibility criteria

The focus of the studies is about IRE with rectangular pulses and a pulse length \( t_p \geq 50 \times 10^{-9} \text{ s} \). They must include analytical and/or numerical analysis of IRE and its heat-generating effect, performed in mammalian subject at macroscopic scale (i.e. tissue, scaffolds, cell suspension, phantoms including mammalian cell suspension). The inclusion criterion in the meta-analysis was the inclusion of a calculated temperature increase with the corresponding pulse parameters, and electrical and thermal properties of the target tissue. Studies that do not satisfy the criterion of the meta-analysis, or studies with the focus on the combination of IRE with other electroporation treatments (e.g. reversible electroporation, high-frequency IRE) or thermal ablations were included in qualitative synthesis.

Studies were excluded, if: (1) they exclude analytical and/or numerical analysis of the heat-generating effect of IRE, and the electrical properties of the target subject are not reported, (2) they applied IRE to nonmammalian subject, (3) they only include analytical and/or numerical analysis at microscopic scale (i.e. single cell, multiple cells, planar lipid membrane), (4) \( t_p < 50 \times 10^{-9} \text{ s} \) or the IRE pulses are nonrectangular, (5) their focus related to general description of electroporation, pulses with low electric field strength, reversible electroporation (including agent/drug/gene delivery) or thermal ablation, (6) they are a review, (7) their focus is about design of pulse generator.
2.3. Data collection

Parameters and equations, which were used for the analytical and/or numerical analysis of IRE and its heat-generating effect, were extracted from studies included in qualitative synthesis. Data of the included studies were extracted by one author (PA) and checked by a second author (EvV). Any disagreement was resolved by discussion between the review authors. Abbreviations, parameters, quantities, and study characteristics extracted from the included studies were explained in Supplementary Appendix 2. The extracted equations were reviewed and summarized in Section 3 and extensively described in Supplementary Appendix 3. After explaining the analysis of certain study characteristics in Supplementary Appendix 5 (see Subsection 2.4), we summarized the extracted parameters and study characteristics in four tables that are shown in Supplementary Appendix 6.

2.4. Methods of analysis

2.4.1. Definitions of mild-hyperthermic and thermally ablative effects

Assuming the tissues to have an initial physiological temperature \( T_{\text{init}} \) of \( 37 \) °C and a thermally ablative threshold \( T_{\text{th}} \) of \( 50 \) °C \( \geq \Delta T \geq 13 \) °C, the following assumptions were made:

- The thermal elevation of \( 3 \leq \Delta T \leq 13 \) °C \( (40 \leq T \leq 50) \) results in mild-hyperthermic effects.
- The thermal elevation of \( \Delta T \geq 13 \) °C \( (T \geq 50) \) results in thermally ablative effects.
- Accordingly, in the IRE-TR the relative sizes were calculated based on the regions with the thermal elevations of \( 3 \leq \Delta T \leq 13 \) °C for the mild-hyperthermic effects and \( \Delta T \geq 13 \) °C for the thermally ablative effects.

Please note that even though the value 50 °C may not instantaneously result in thermal damage, it was still chosen as a \( T_{\text{th}} \). This because during IRE the duration of exposure to temperatures around 50 °C could be in the order of minutes \([15,16]\), which is sufficiently long that thermal damage may indeed occur \([14,23]\) and this is thus effectively a clinically relevant threshold value associated with a high likelihood of occurrence of thermal ablation.

2.4.2. Analysis of research questions

To answer the first two research questions, we extracted the maximum simulated temperature increase \( \Delta T_{\text{max}} \) with the corresponding pulse parameters from studies included in meta-analysis. If the data extracted from the pulse parameters and/or the maximum simulated temperature was incomplete, the \( \Delta T_{\text{max}} \) was not reported. In case of parametric study, in which \( \Delta T_{\text{max}} \) was produced from several pulse parameters, the \( \Delta T_{\text{max}} \) at the extreme ends of the figures were included.

For the third research question, if a study included 2D plots of electric field, and temperature distributions at the
same position of the same model, then \( S_{3AT13,S} \) (\( \text{m}^2 \)), \( S_{AT13,S} \) (\( \text{m}^2 \)), and \( S_{EIRE(th),S} \) (\( \text{m}^2 \)) were extracted, where \( S_{3AT13,S} \) is the total surface area of the simulated temperature increase within the range \( 3 \leq \Delta T \) [\(^\circ\text{C}\)] \( < 13 \) that represents the region with mild-hyperthermic temperature increase, \( S_{AT13,S} \) is the total surface area of the simulated temperature increase with \( \Delta T \geq 13 \) [\(^\circ\text{C}\)] that represents the thermally ablated region, and \( S_{EIRE(th),S} \) is the total surface area of the simulated electric field with \( E \geq EIRE(th) \) that represents the IRE-TR. \( E \) (\( \text{V m}^{-1} \)) is the magnitude of the electric-field strength and \( EIRE(th) \) (\( \text{V m}^{-1} \)) is the electric-field threshold of IRE; the minimum \( E \) required to ablate the target tissue. Even though \( EIRE(th) \) was only used as a minimum required \( E \) to extract \( S_{EIRE(th),S} \) for this analysis, it must be noticed that \( EIRE(th) \) in reality also depends on pulse parameters (pulse voltage, pulse shape, pulse length, pulse number, and pulse frequency), temperature, and electrical conductivity of the target. It is also important to note that:

- the \( EIRE(th) \) values correspond to the \( EIRE(th) \) from the study from which \( S_{EIRE(th),S} \) was extracted,
- the pulse parameters on which the values of \( \Delta T_{max} \) \( S_{3AT13,S} \) and \( S_{AT13,S} \) depend, are different for each study,
- in case of \( EIRE(th) \) range, e.g. \( [EIRE(th),1, EIRE(th),2] \), the average of this range was used to calculate \( S_{EIRE(th),S} \). Specifically, \( EIRE(th) = \frac{1}{2} (EIRE(th),1 + EIRE(th),2) \).
- if \( EIRE(th) \) was not reported in a certain study, then the \( S_{EIRE(th),S} \) was not calculated and, accordingly, included in the analysis.

The extraction of the surface areas was done excluding surface areas of the electrodes (\( S_{elect} \) (\( \text{m}^2 \))). Using the software package ImageJ (National Institutes of Health, Bethesda, MD), we firstly imported the desired figure in ImageJ and assigned the pixels to distance ratio of the image using the known distance between the electrodes. Next, if the imported figure includes \( E- \Delta T \)-contours showing the conditions mentioned above, then we manually selected \( S_E \) (\( \text{m}^2 \)), \( S_{AT} \) (\( \text{m}^2 \)) and \( S_{elect} \) using the ‘Freehand selections’ tool; \( S_E \) is the selected surface area of the electric field strength and \( S_{AT} \) is the selected surface area of the temperature increase. In case of no contours, we used the ‘Threshold Color’ tool to set the threshold of the surface color to the minimum value mentioned above. Circular or rectangular electrodes were manually selected using ‘Oval selections’ tool, and ‘Rectangle’ tool. After the extraction, the ratios \( R_{3AT13} \) (%) and \( R_{AT13} \) (%) were calculated, where \( R_{3AT13} \) is the ratio between the region with mild-hyperthermic temperature increase and IRE-TR, and \( R_{AT13} \) is the ratio between the thermally ablated region and IRE-TR. The accurate description of the extracted surface areas and calculated ratios are shown in Supplementary Appendix 5.

### 2.5. Statistical analysis

Statistical analysis was applied to the data of the studies included in the meta-analysis to answer the three research questions. Specifically, we answered the first two research questions by calculating the median, the minimum, and the maximum of \( \Delta T_{max} \). For the third research question, the medians were calculated of \( R_{3AT13} \) and \( R_{AT13} \). The answers of all research questions were based on studies with experimentally applied pulse parameters. As an additional step, the linear regression was applied to model the relationship between \( S_{EIRE(th)}, R_{3AT13} \), and \( R_{AT13} \). And the energy density (\( \text{u} [\text{J} \cdot \text{m}^{-3}] \)); see Subsection 3.3), \( \Delta T_{max} \) \( R_{3AT13} \), and \( R_{AT13} \).

#### 2.6. Additional assumptions, definitions, methods of analysis, and notes

To preserve the continuous course, all remaining assumptions, definitions, methods of analysis, and notes were defined in Section 3 when the need arose.

### 3. Results

#### 3.1. Study selection

After removal of duplicates and screening 3815 articles by title, abstract, and full-text, we included 58 articles [3,24–80] in the qualitative synthesis, and 26 articles [3,24–28,31,33,34,36,37,39–44,48,49,55,59,60,64,66,73,80] in the quantitative synthesis (meta-analysis and meta-regression). The full overview of the study selection is shown in Figure 1. The parameters extracted from the included studies are shown in Supplementary Appendix 6, with the definitions explained in Supplementary Appendix 2.

#### 3.2. Review of mathematical description of IRE

In this section, we summarized and reviewed the mathematical equations used to describe the thermal behavior of IRE in multiple models with various types of model geometries, electrode, pulse parameters, tissue properties, and boundary conditions. The extended description of the mathematical equations can be found in Supplementary Appendix 3. In addition, more details about the applied model parameters were included in Supplementary Appendix 6. In the included studies, the subjects were modeled in one-dimensional [25,30,50,64], twodimensional [3,24–28,32–34,36–39,41,51–54,59,65,66,72,74,75,78], and/or three-dimensional space [26,29,31,35,40,42–49,55–58,60–63,67–71,73,77,79,80]. The calculations were performed analytically, or numerically using Finite Element Method or Finite Difference Method using the software packages e.g. CFDdesign (Blue Ridge Numerics, Inc., Charlottesville, VA) [55], COMSOL Multiphysics (COMSOL AB, Stockholm, Sweden) [26,28,31,32,34–46,48,49,51–53,56–63,65–67,70,71,73,74,76–78], FEMLAB (COMSOL AB, Stockholm, Sweden) [3,24,27], IRENA [79,81], Marc/Mentat (MSC Software Corp., Santa Ana, CA) [47,75], and QuickField (Terra Analysis, Denmark) [54]. Generally, numerical calculations were performed on three-dimensional subjects using the software package COMSOL Multiphysics, which uses Finite Element Method. Furthermore, an example of possible pulse sequence is shown in Figure 2. Here, \( V_p \) [\( \text{V} \)] is the electric potential (the voltage) of the pulses, \( t_p \) [\( \text{s} \)] is the duration of
a single pulse, \( n_p \) [-] is the pulse number, \( N_p \) [-] is the total number of pulses, \( \tau_p \) [s] is the duration between two pulses, and \( f_p \) [Hz] is the pulse frequency, which is calculated as

\[
f_p = \frac{1}{t_p + \tau_p} \tag{1}
\]

For convenience, overview of definitions, symbols, and mathematical notations were summarized in Supplementary Appendix 2.

### 3.2.1. Electric-field distribution

#### 3.2.1.1. Electric-potential models. The IRE can be mathematically described at macroscopic scale by calculating the electric-field \([3,24,26–29,31,33,34,36–48,50–59,61–63,65,66,68–70,72–75,78–80]\), and temperature distribution \([3,24,26–31,33,34,36,37,39–44,48,53,55,58,59,61,62,64,66,70,73,76,80]\) in a volume of a certain medium (e.g. tissue). This description starts with the calculation of the electric-potential distribution, which is obtained by simplifying the combination of Maxwell’s equations: Faraday’s law of induction and Ampère’s law, into the steady-state form

\[
\nabla \cdot (\sigma \nabla \Phi) = 0 \tag{2}
\]

where \( \nabla \cdot [\text{m}^{-1}] \) is the divergence, \( \sigma [\text{S} \cdot \text{m}^{-1}] \) is the electrical conductivity, \( \nabla [\text{m}^{-1}] \) is the gradient and \( \Phi [\text{V}] \) is the scalar electric potential \([3,24,26,27,29,31,33,34,36–48,50–59,62,63,65,66,68–70,75,78–80]\). Here, the vector quantities are expressed in bold and italic, while the scalar quantities have remained unchanged. Furthermore, the electrical conductivity of a medium can be assumed to be homogeneous, heterogeneous, isotropic, anisotropic, linear, and/or nonlinear (see Supplementary Appendix 2 Table A2.2 for the definitions of the medium properties and Supplementary Appendix 3 for extended description of medium properties). This electrical model can be used when several media are modeled with various electrical characteristics, such as cancerous tissue in a healthy tissue model, and tissue that includes blood vessels and nerves. Also, it is possible to choose a volume with homogenous, isotropic, and linear medium properties for simplicity. In this case, we can remove the electrical-conductivity term from Equation (2) and transform it into the Laplace’s equation

\[
\nabla^2 \Phi = 0 \tag{3}
\]

with \( \nabla^2 [\text{m}^{-2}] \) as Laplace operator.

3.2.1.2. Electric-field model and threshold. For both Equations 2 and 3, the calculated electric-potential distribution is used to determine the electric-field distribution in the volume of the medium using

\[
E = -\nabla \Phi \tag{4}
\]

with \( E [\text{V} \cdot \text{m}^{-1}] \) as the electric field \([26,43,68,72,74,79,80]\). Assuming the volume of the medium to be an arbitrary tissue, the electric-field distribution yields IRE when a minimum electric-field strength \( E_{\text{IRE(th)}} \) is obtained, providing the corresponding duration, number, shape, and the frequency of the pulses, and the tissue type. In Supplementary Appendix 6, a range of \( E_{\text{IRE(th)}} \) and its corresponding pulse parameters are shown for several tissue types. Additional details about the mathematical description of the electric-field component of IRE are shown in Supplementary Appendix 3.

3.2.1.3. Boundary conditions. For the calculation of the electric potential, a boundary condition (BC) needs to be applied to the boundaries between the electrodes (metallic part) and the media (BEM), and the boundaries at the outer surface (BOS) of the model. These boundaries could have a constant electric potential (Dirichlet BC; always applied by the included studies to BEM) \([3,24,26–29,31–48,50–55,57–61,64–66,68–76,78–80]\), zero electric current (Neumann BC; generally applied to BOS) \([3,24,26,28,29,31–34,36–45,48,53,57–61,66,69,70,73–75,78–80]\), or limited electric current to reduce the influence of the boundaries on the domain by virtually increasing the size of the domain while maintaining the number of nodes (Robin BC) \([79]\). See Figure 3.

The preference of the boundary conditions depends on the type of the study. In case of active electrodes, obviously Dirichlet BC should be applied, while Neumann BC could be applied to the nonmetallic parts of the electrodes. For BOS, it is recommended to apply the Neumann BC for the clinical
practice, since the simulated electric field can provide an upper limit to the temperature distribution [3].

3.2.2. Temperature distribution

3.2.2.1. Bioheat transfer models. Several heat transfer models were used for the calculation of thermal distributions (see Supplementary Appendix 3 for an extended summary). Nevertheless, the most utilized model is the Pennes bioheat equation

\[
\rho c_p \frac{\partial T}{\partial t} = \nabla \cdot (k \nabla T) - \rho_b \omega_b c_b (T - T_{\text{art}}) + Q_m + J \cdot E
\]  

where \( \rho \) (kg m\(^{-3}\)) is the mass density, \( c_p \) (J kg\(^{-1}\)C\(^{-1}\)) is the specific heat capacity, \( T \) (C) is the temperature, \( t \) [s] is the time, \( k \) (W m\(^{-1}\)C\(^{-1}\)) is the thermal conductivity, \( c_b \) (J kg\(^{-1}\)C\(^{-1}\)) is the specific heat capacity of the blood, \( \rho_b \) (kg m\(^{-3}\)) is the blood density, \( \omega_b \) [s\(^{-1}\)] is the blood perfusion rate (blood perfusion \( \omega_b \) (kg m\(^{-3}\)s\(^{-1}\)) = \( \rho_b \cdot \omega_b \)), \( T_{\text{art}} \) [C] is the arterial blood temperature, \( Q_m \) (W m\(^{-3}\)) is the metabolic heat generation, and \( J \cdot E \) (W m\(^{-3}\)) is the heat generation rate per unit volume (the Joule Heating) [3,24,26–29,31,33,34,36,37,39,41–44,48,53,55,58,61,62,66,70,73,76,80]. Over the intrapulses of IRE, the Joule Heating term can be simplified into

\[
J \cdot E = \sigma |E|^2
\]  

where IRE is used [3,24,26–29,31,33,34,36,40,41,44,48,53,55,58,59,66,76].

Even though the majority of the studies applied the Pennes bioheat model, the choice of a suitable heat transfer model depends on the modeling conditions. Yet, for the clinical practice the Pennes bioheat model is recommended due to its ability to calculate the temperature distribution in a domain with heterogeneous and nonlinear electrical and thermal characteristics, considering the metabolic heat generation and heat sink effect.
where $\Delta T$ [°C] is the temperature increase during the time period $\Delta t$ [s] and $t_p \cdot f_p$ is a dimensionless factor that averages the Joule Heating term over the entire intra- and inter-pulse duration (see Supplementary Appendix 3). For illustration, Figure 5(B) shows the temperature profile of Equation 7 as a function of time over the period $t_{end} - t_{init}$ (or $N_p \cdot (t_p + t_f)$) with normothermic (from $t_{init}$ to $t_{MH}$), mildly-hyperthermic (from $t_{MH}$ to $t_{TA}$) and thermally ablative zones (from $t_{TA}$ to $t_{end}$). According to this figure, the ratios can be calculated by

$$R\Delta t_{MH} = \frac{\Delta t_{MH}}{N_p \cdot (t_p + t_f)}$$

and

$$R\Delta t_{TA} = \frac{\Delta t_{TA}}{N_p \cdot (t_p + t_f)}$$

where $R\Delta t_{MH}$ and $R\Delta t_{TA}$ are dimensionless ratios between mild-hyperthermic and thermally ablative time durations, and the total treatment time. Due to the linearity of Figure 5(B),

$$\frac{\Delta t}{N_p \cdot (t_p + t_f)} = \frac{\Delta T}{\Delta T_{max}}$$

After combining Equations 8 and 9 with 10, the time durations can be replaced by the temperature differences as follows

$$R\Delta t_{MH} = \frac{\Delta T_{MH}}{\Delta T_{max}}$$

with

$$\Delta T_{MH} \in \{ \Delta T - 3 \leq \Delta T < 13 \}$$

and

$$R\Delta t_{TA} = \frac{\Delta T_{TA}}{\Delta T_{max}}$$

with

$$\Delta T_{TA} \in \{ \Delta T - 13 \mid \Delta T \geq 13 \}$$

Since the required data to calculate the total treatment time for each study are available, the calculation of $R\Delta t_{MH}$ and $R\Delta t_{TA}$ allows us to estimate the time durations of mild hyperthermia and thermal ablation using Equations 8 and 9.

### 3.2.3. Thermal damage

Exposing biological cells or tissues to temperatures higher than their physiological temperatures for extended periods of time can result in thermal damage. In other words, irreversible changes in tissue structure or function that includes cell death, microvascular blood flow stasis, and/or protein coagulation processes [40]. The thermal damage starts at the mild-hyperthermic temperature 42°C–43°C with exposure period between several seconds to hours, depending on the temperature level [40,53,58]. In the case of relatively short exposure, damage is relatively limited up to 50°C–60°C, at which level the damage dramatically increases [40,58]. For instance, 73.4°C is considered the threshold for instantaneous thermal damage in liver tissue that results in tissue whitening [53]. Nevertheless, 50°C could be generally assumed as a typical and relevant threshold one should attempt to avoid in the clinic as when temperatures around 50°C occur during IRE the duration of exposure to these elevated temperatures could be in order of minutes [15,16], with a significant likelihood of resulting in thermal damage [14,23]. Multiple studies quantified the amount of thermal damage using the Arrhenius equation [3,26,28,30,33,34,36,40,41,53,55,58,59,80]. This equation is a first-order reaction rate constant that describes the rate of a chemical reaction. The Arrhenius equation, firstly applied by Henriques and Moritz in 1947 for skin burn analysis in pigs [18,82–84], uses Maxwell–Boltzmann statistics to describe the conversion of biological substances at temperature $T_k(t)$ from a native state to a thermally damaged state at a rate constant

$$\frac{\partial \Omega(t)}{\partial t} = A \exp\left(-\frac{U_a}{RT_k(t)}\right)$$

where $\Omega(t)$ is the dimensionless time dependent accumulated thermal damage, $A$ [s$^{-1}$] is the pre-exponential factor (or collision frequency), a measure for molecular collision frequency, $U_a$ [J·mol$^{-1}$] is the activation energy required for the molecules to denature, $R$ [J·mol$^{-1}$·K$^{-1}$] is the ideal gas constant ($R = 8.314$ [J·mol$^{-1}$·K$^{-1}$]), and $T_k(t)$ [K] is the time-dependent absolute temperature [80]. Then, the thermal damage is calculated by integrating the Arrhenius Equation over a certain period of time, resulting in

$$\Omega(t) = \int_{t_{init}}^{t} A \exp\left(-\frac{U_a}{RT_k(t)}\right) dt$$

where $t_{init}$ [s] is the initial time point of the heating process [3,26,28,30,33,34,36,40,41,53,55,58,59,80]. The power of this analysis is to provide an estimation of the damaged fraction of a certain volume of a tissue constituent that is exposed to temperature $T_k$. This estimation is calculated by hypothetically expressing both the intact and damaged substances in

$$\Omega(t) = \ln\left(\frac{N(t_{init})}{N(t)}\right)$$

where $N(t_{init})$ and $N(t)$ represent the amount of the substances (expressed in number or concentration) in the tissue volume just before the temperature exposure and at time $t$ [33,55,59]. Then, the probability of thermal damage (the estimation of the damaged fraction), Y [%], in the volume of the tissue constituent is calculated [33,53,55,59,80] using

$$Y = 100\left(1 - \exp(-\Omega(t))\right)$$

The severeness of injuries due to the heating process can be represented by a threshold $\Omega_{th}$. For instance, it has been shown that the threshold for burn injuries in blood-perfused skin is $\Omega_{th} = 0.53$ (Y = 41%) while it increases to $\Omega_{th} = 10,000$ (Y = 100%) for third-degree burns [40,58]. In order to describe the thermal damage processes, such as cell death, microvascular blood flow stasis, or protein coagulation, suitable A and $U_a$ values must be chosen for each process, which are tissue specific and experimentally determined [53].
Examples of such values can be found in Supplementary Appendix 6.

Another approach to describe the thermal damage is to calculate the cumulative equivalent minutes at 43 °C (CEM43 °C [min]; also defined as the thermal isoeffect dose \( t_{\text{th}} \) [min]) \[25,26,29,31,58,76\]. CEM43 °C is a concept, introduced by Sapareto and Dewey \[19\], which scales the exposure time of a time-varying temperature to an equivalent exposure time at the reference temperature 43 °C. Specifically, during the equivalent exposure time the tissue is assumed to be constantly held at 43 °C. CEM43 °C can be described as

\[
\text{CEM43 °C} = \int_{t_{\text{init}}}^{t} R^{33} - \text{dt}
\]

where \( t_{\text{dt}} \) is the average temperature over the time differential \( \text{dt} \), \( R \) is the factor to compensate for a 1 °C temperature change with \( R = 0.25 \) when \( t_{\text{dt}} \leq 43 °C \) and \( R = 0.5 \) when \( t_{\text{dt}} > 43 °C \). In this case, the severity of injuries due to the heating process can be represented by a threshold CEM43 °C\(_{\text{th}}\). Examples of such values can be found in Supplementary Appendix 6 and \[85,86\].

The use of a suitable thermal damage model depends on both the temperature range to which the target had been exposed to, and the desired expression of the thermal damage outcome. E.g., CEM43 °C can provide a reasonable prediction of cell death for the temperature range 40 °C–47 °C \[87\]. However, it does not provide the probability of thermal damage as in the Arrhenius equation. On the other hand, the Arrhenius equation may overestimate the cell death at the start of the mild-hyperthermic temperature range \[88\]. More information about both models can be found in \[19,88,89\].

### 3.2.4. Tissue properties

The electric-field and temperature distributions of IRE have been calculated for multiple tissue types. For example, IRE has been applied to artery \[33,34,36,41\], brain \[31,40,57,66,69,73\], breast \[28,29,35\], cervix \[78\], eye \[44\], in vitro cell suspension \[30,43,47,60,61,75\], kidney \[45,63,65\], liver \[3,24,37–39,46,48,53,54,62,71,72,74,76,77,79,80\], pancreas \[52,70\], prostate \[28,32,49,50,56,64,67,68\], rectal wall \[51\], skin \[25,58\], small intestine \[59\], and subcutaneous tissue \[42\]. Although some studies used the same tissue type, often different tissue properties were applied, which were classified as homogeneous or heterogeneous, isotropic or anisotropic, and linear or nonlinear. The definitions of these terms can be found in Supplementary Appendix 2 Table A2.2. For instance, the tissue properties were generally assumed to be isotropic linear, e.g. \( \sigma, \rho, \varepsilon, K, Q_{\text{mv}}, w_{\text{p}}, A, \text{and } U_{\text{v}} \). Specifically, this was the case for \( \rho, \varepsilon, Q_{\text{mv}}, w_{\text{p}}, A, \text{and } U_{\text{v}} \) in all of the included studies. However, some studies assumed isotropic nonlinear tissue properties, e.g. \( \sigma(E) \) \[42,45,53,54,56,57,59,63,66,68–71,77,79\], \( \sigma(T) \) \[3,40,43,45,60\], \( \alpha(E,T) \) \[31,37,40,45,62,73,76,80\], and \( K(T) \) \[3\]. In particular, most of the studies fitted the electrical conductivity in the form

\[
\sigma(E) = \sigma_{\text{init}} + (\sigma_{\text{max}} - \sigma_{\text{init}}) \frac{1}{1 + a_1 \exp \left( \frac{-E - a_2}{a_3} \right)}
\]

with

\[
a_2 = \frac{E_{\text{RE(th)}} + E_{\text{RE(th)}}}{2}
\]

and

\[
a_3 = \frac{E_{\text{RE(th)}} - E_{\text{RE(th)}}}{a_4}
\]

where \( \sigma_{\text{init}} [\text{S} \cdot \text{m}^{-1}] \) is the initial electrical conductivity of the target medium before application of IRE, \( \sigma_{\text{max}} [\text{S} \cdot \text{m}^{-1}] \) is the electrical conductivity of the maximally permeabilized medium, and \( a_1, a_2, \text{and } a_3 \) are the sigmoid function parameters. Additional detail about nonlinear electrical conductivities is provided in Supplementary Appendix 3.

The majority of the included studies applied a static (constant) electrical conductivity to calculate the electric field distribution. However, as shown by \[90,91\] for example, the electrical conductivity has a dynamic behavior due to its increase during the electroporation. By including a dynamic electrical conductivity, the numerical models can be better correlated to the experimental data, such as the electric current, as shown by \[63,92\]. In addition, the inclusion of the dynamic behavior can reduce the variability of \( E_{\text{RE(th)}} \) as was demonstrated by \[63\] in canine kidneys \((575 \times 10^2 \pm 67 \text{ V} \cdot \text{m}^{-1}); \text{ versus } 501 \times 10^2 \pm 121 \times 10^2 \text{ V} \cdot \text{m}^{-1}\) obtained from the model with static electrical conductivity resulting in a proper prediction of \( E_{\text{RE(th)}} \). An additional effect of the dynamically increasing electrical conductivity is the corresponding increase of the electric-field strength as was demonstrated by \[63,68\]. A larger electrical conductivity and electric-field strength result in a larger temperature increase (see Equations 5 and 6). To determine the thermal effects and to properly estimate the possible lesion size of the treatment, it is important to include the dynamic behavior of the electrical conductivity in the treatment planning.

### 3.3. Synthesis of data, meta-analysis, and meta-regression

This section summarizes the extracted data from the included studies, focusing on the maximum simulated temperature increase, and the energy density that was calculated as

\[
u = \sigma_{\text{init}} \frac{\left| V_p \right|^2}{D} t_{\text{th}} N_p
\]

with \( u [\text{J} \cdot \text{m}^{-3}] \) as the energy density, and \( D [\text{m}] \) as the distance between electrodes (see Figure 6). Important: Please note that the \( u \) of an included study is not plotted if one of the extracted parameters in Equation 23 is missing.

#### 3.3.1. Organ-dependent electrical and thermal properties

The calculations of \( \Delta T_{\text{max}} \) \( \eta_{\text{AT13}}, \) \( \eta_{\text{AT13}}, \text{ and } u \) are based on organ-dependent electrical and thermal characteristics, such as \( E_{\text{RE(th)}} \), \( \sigma \), and other properties provided in Equation 5. For these characteristics, a range of selected values was
summarized in Table 1 and plotted in Figure 7 both for cancerous and healthy tissues. Please note that $k_{\text{init}}$ [W m$^{-1}$ K$^{-1}$] represents the initial thermal conductivity before the start of the heating process. To repeat, the electrical and thermal characteristics extracted from the included studies are provided in Supplementary Appendix 6 Table A6.2. Additional electrical and thermal characteristics can be found in the ITIS database for thermal and electromagnetic parameters of biological tissues [93].

### 3.3.2. Thermal effects as function of IRE settings

In Figure 8(A), we evaluated $\Delta T_{\text{max}}$ and $u$ obtained from the reported pulse parameters in the included studies, while in...
Figure 7. Parameters of different tissue types, as selected by the included studies (Supplementary Appendix 6 – Table A6.2). For (A)–(E), red circles represent cancerous tissue and green diamonds represent healthy tissue. In all figures, brain tissue represents gray and white matter, skin tissue represents subcutaneous tissue, fat layer, dermis and stratum epidermis, eye tissue represents aqueous, cornea, lens, retina, sclera and vitreous, colon tissue represents both colon and rectal wall, blood vessel tissue represents artery, blood and blood vessel, and finally, muscle tissue represents muscles with longitudinal and transversal properties. The selected tissue properties in this figure show (A) the initial electrical conductivity ($\sigma_{\text{init}}$ [S/m]) before the application of IRE (the $\sigma_{\text{init}}$ values that exceeded 1.5 S/m were 1.5 S/m for aqueous and vitreous in the eye [44], and 2.31 S/m for cancerous breast tissue [28]), (B) mass density ($\rho$ [kg/m$^3$]), (C) specific heat capacity ($c_p$ [J/kg$^\circ$C]), (D) initial thermal conductivity before the start of heating process ($k_{\text{init}}$ [W/m$^\circ$C]), (E) blood perfusion ($w_b$ [kg/m$^3$s$^{-1}$]; the $w_b$ value that exceeded 20 kg/m$^3$s$^{-1}$ was 212 kg/m$^3$s$^{-1}$ for both healthy and cancerous pancreas tissues [70]; if $w_b$ could not be extracted nor calculated due to the absence of blood density ($\rho_b$ [kg/m$^3$]), then $w_b$ was calculated by assuming $\rho_b = 1060$ kg/m$^3$), and (F) Electric-field threshold of irreversible electroporation ($E_{\text{IREN}}$ [V/m$^{-1}$]) as function of pulse number. Please note that the y-axis in (F) can be read in [V cm$^{-1}$], and that empty fields are present in (B)–(F) due to the lack of data.
Figure 7. Continued.
Figure 8(B) we only considered the more realistic subset of experiments that were actually applied in experiments. Furthermore, Figure 8(A) shows that the pulses produce $\text{MEDIAN} (\Delta T_{\text{max}}) = 9.25 \degree C$ with the range $0.27 \leq \Delta T_{\text{max}} [\degree C] \leq 135$, and $\text{MEDIAN}(u) \approx 0.37 \times 10^6 \text{ J m}^{-3}$ with the range $1.19 \times 10^5 \leq u [\text{J m}^{-3}] \leq 11.42 \times 10^5$. In addition, Figure 8(B) shows that experimentally applied pulses produce $\text{MEDIAN} (\Delta T_{\text{max}}) = 9.49 \degree C$ with the range $1 \leq \Delta T_{\text{max}} [\degree C] \leq 39$, and $\text{MEDIAN}(u) = 0.81 \times 10^6 \text{ J m}^{-3}$ with the range $2.92 \times 10^5 \leq u [\text{J m}^{-3}] \leq 8.22 \times 10^6$. In a similar way, we presented $\Delta T_{\text{max}}$ and $u$ in Figure 8(C) as in Figure 8(B), with the exception of having ‘Total treatment time ($N_p \cdot f_p^{-1}$ [s])’ instead of ‘Total pulsing time’ with a $\text{MEDIAN}(\text{Total treatment time}) = 80 \text{ s}$ with the range of $12.5 \leq \text{Total treatment time} [\text{s}] \leq 540$ ($0.21 \leq \text{Total treatment time} [\text{min}] \leq 9$).

Assumption: In case of multiple target tissues, e.g. the heterogeneous small intestine [59], the tissue in the center of the volume was assumed to be the target.

### 3.3.3. Thermal effects as function of irreversible permeabilization effect

Furthermore, we presented the ratios between the mild-hyperthermic and thermally ablative effects, and IRE-TR, $R_{3\text{AT}13}$ and $R_{\text{AT}13}$, as a function of $S_{\text{SE-IRE(th)}}$ in Figure 9. Again, here we included $\Delta T_{\text{max}}$ and $u$ of the experimentally applied pulse parameters. Despite the small number of data points, this figure shows that an increase in the IRE-TR results in a constant mild-hyperthermic effects with $\text{MEDIAN}(R_{3\text{AT}13}) = 31.75\%$ and the range $0 \leq R_{3\text{AT}13} [\%] \leq 88.67$. In addition, it also results in the increase of the thermally ablative effects with $\text{MEDIAN}(R_{\text{AT}13}) = 4.57\%$ with the range of $0 \leq R_{\text{AT}13} [\%] \leq 41.3$.

### 3.3.4. Thermal effects as function of energy density

The data obtained from Figures 8(C) and 9 were used in Figure 10 to show the relationships between $u$ and $\Delta T_{\text{max}}$ and $u$, $R_{3\text{AT}13}$ and $R_{\text{AT}13}$. These figures indicate that an increase in the $u$ results in an increase of $\Delta T_{\text{max}}$ and $R_{3\text{AT}13}$; while $R_{\text{AT}13}$ remains almost constant.

### 3.3.5. Estimation of time durations of thermal effects

Figure 11 illustrates the estimated time durations of both mild-hyperthermic and thermally ablative temperatures during IRE at the spatial point with the maximally simulated temperature increase $\Delta T_{\text{max}}$. This was done for the studies included in meta-analysis with experimentally applied pulses. Please note that $\Delta T_{\text{MH}}$ and $\Delta T_{\text{TA}}$ were not calculated if they were provided by the included studies. Also, the data of $\Delta T_{\text{MH}}$ and $\Delta T_{\text{TA}}$ after the IRE treatment were included in Figure 11 only if they were provided by the included studies. To repeat, $\Delta T_{\text{MH}}$ and $\Delta T_{\text{TA}}$ represent the time durations of $3 \leq \Delta T_{\text{max}} [\degree C] \leq 13$ (mild hyperthermia) and $\Delta T_{\text{max}} [\degree C] \geq 13$ (thermal ablation). The condition ‘$\Delta T_{\text{max}} [\degree C] \geq 13$’ at the spatial location with maximal simulated temperature increase (see Figure 5) indicates the presence of mild hyperthermia for a duration of $\Delta T_{\text{MH}}$ prior to thermal ablation. Therefore, if the data from the included studies met the condition ‘$\Delta T_{\text{max}} [\degree C] \geq 13$’, then both values of $\Delta T_{\text{MH}}$ and $\Delta T_{\text{TA}}$ are plotted. Figure 11 shows that during the IRE treatment, the time duration for mild hyperthermia was $\text{MEDIAN} (\Delta T_{\text{MH}}) = 15 \text{ s}$ with $0 \leq \Delta T_{\text{MH}} [\text{s}] \leq 100$, while the time duration for thermal ablation was $\text{MEDIAN} (\Delta T_{\text{TA}}) = 0 \text{ s}$ with $0 \leq \Delta T_{\text{TA}} [\text{s}] \leq 133.3$. The median values include both calculated and extracted values. Furthermore, the studies from which $\Delta T_{\text{MH}}$ and $\Delta T_{\text{TA}}$ were extracted during and after IRE had medians of $\text{MEDIAN}(\Delta T_{\text{MH}}) = 0 \text{ s}$ with $0 \leq \Delta T_{\text{MH}} [\text{s}] \leq 180.7$ and $\text{MEDIAN}(\Delta T_{\text{TA}}) = 0 \text{ s}$ with $0 \leq \Delta T_{\text{TA}} [\text{s}] \leq 44.9$.

### 4. Discussion

#### 4.1. Research questions

According to the predictions of the numerical models, the relative sizes of both locally mild-hyperthermic and thermally ablative effects in the IRE-TR, with respect to the IRE-TR, have approximate medians of 30 and 5%, respectively. Accordingly, IRE produces sufficient heat to thermally ablate small parts of the IRE-TR, and to raise the temperature within the range of $40 \leq T [\degree C] < 50$ in a third of the IRE-TR. This temperature range probably reduces the $E_{\text{IRE(th)}}$ of the tissues and, consequently, enhances the IPE as was shown ex vivo for $43 \degree C$ in [20]. Mouse ectopic pancreatic cancer treated using an integrated IRE system with controllable laser heating at $42 \degree C$ was compared with standard IRE without additional heating. A complete tumor regression was observed in more than 50% of the tumor-bearing mice, while in the standard IRE group no complete regression was observed [19]. Results of this review predict that the IPE including the mild-hyperthermic effect dominates the IRE-TR with an approximate median of 95% (30% by combined contributions of mild-hyperthermic and IPE effects, and 65% by IPE effect only), implying that the IRE is based on the IPE with sufficient heat as adjuvant to enhance this effect. Again, it is important to notice that: first, the $E_{\text{IRE(th)}}$ values in this review are different for each study, these medians attempt to mimic the real world by utilizing dynamic electrical conductivity and perfusion. In addition, a Neumann boundary condition was mostly used for the electrical and thermal outer boundary conditions to estimate the upper limit of the induced temperatures. Therefore, these medians are likely to represent the thermal effects on the safe side.

The calculations of these medians were based on treated regions simulated by various types of setups. Specifically, the choice of the modeling parameters such as electrode type, electrical and thermal properties, and the type of BC can result in a different outcome of $R_{3\text{AT}13}$ and $R_{\text{AT}13}$. Nevertheless, most of the studies used for the calculation of these medians attempt to mimic the real world by utilizing dynamic electrical conductivity and perfusion. In addition, a Neumann boundary condition was mostly used for the electrical and thermal outer boundary conditions to estimate the upper limit of the induced temperatures. Therefore, these medians are likely to represent the thermal effects on the safe side.

The maximum temperature increase in Figure 8(A) shows that the pulses produce $\text{MEDIAN}(\Delta T_{\text{max}}) = 9.25 \degree C$ with $0.27 \leq \Delta T_{\text{max}} [\degree C] \leq 135$, but the exact predicted maximum temperature depends on the pulse parameters and other
Figure 8. (A) The study characteristics ‘Tissue type’, ‘ΔTmax’, ‘σmax’, ‘Electrode type’, and ‘u’ were plotted as function of ‘Pulse voltage (VP [V])’ and ‘Total pulsing time (tP/C1 NP [s])’ in logarithmic scale. For a proper clarification of the data points, an extended version can be found in Supplementary Appendix 4 Figure A4.1. The range of ΔTmax was 0.27 ≤ ΔTmax ≤ 135, which was represented by the linearly scaled red-yellow color bar with the range 0 < ΔTmax ≤ 40. The ΔTmax values that exceeded 40°C were 50°C at (750 V, 9 x 10⁻³ s) [34], 55°C at (1500 V, 1 x 10⁻² s) [64], 85°C at (2000 V, 3 x 10⁻³ s) [55], and 135°C at (2000 V, 3 x 10⁻⁶ s) [55]. Furthermore, the range of u was 1.19 x 10⁶ ≤ u [J/m³] ≤ 11.42 x 10⁸, which was represented by the linearly scaled blue-magenta color bar with the range 0 < u ≤ 10 x 10⁸ J/m³. The u values that exceeded 10 x 10⁸ J/m³ were two times the value 11.42 x 10⁸ J/m³ at (2000 V, 9 x 10⁻³ s) [44]. (B) Here, we only plotted the data that was obtained from the pulse parameters which were applied in the experiments. The range of ΔTmax was 1 ≤ ΔTmax ≤ 39, and the range of the energy density was 2.92 x 10⁶ ≤ u [J/m³] ≤ 8.22 x 10⁸. (C) is similar to (B), with the exception of having ‘Total treatment time (NP fP/C1 f [s])’ in logarithmic scale instead of ‘Total pulsing time’. Please note the change in Y-axis in (C) in comparison to (B), and that the electrical conductivity is the conductivity before the treatment.
In Figure 8(B,C) we only included factors assumed in the model. One would expect that an increase in the pulse voltage and the total pulsing time would result in an increase of $\Delta T_{\text{max}}$. However, these figures do not show a clear correlation between pulse voltage, pulsing time and temperature increase, since the temperature increase also depends on the pulse frequency (higher pulse frequency results in larger temperature increase) and on the model configuration. An adjustment of these properties results in different $\Delta T_{\text{max}}$. For instance, $\Delta T_{\text{max}}$ including the ratios of the mild-hyperthermic and thermally ablative regions with respect to IRE-TR, increases as a result of the increase of the pulse voltage, the pulse duration, the pulse frequency, the electrical and the thermal conductivity of the tissue and the number of electrode pairs, and by assuming Neumann boundary conditions at the electrode-medium interface and the outer surface of the model. Besides this, other factors yielding a higher predicted maximum temperature are a decrease in the distance between the electrodes, a decrease in the specific heat capacity and density, and exclusion of the blood perfusion term. Example of variation in both electrical and thermal properties is shown in Figure 7(A–E). These figures show differences between the values, even within the same tissue category.

The majority of the studies in Figure 8(A) did not actually apply the simulated pulses in experiments. In fact, some authors performed primarily a parametric study in which pulse, setup or tissue characteristics were varied, resulting in occasionally very large $\Delta T_{\text{max}}$ values [34,39,44,55]. Therefore, in Figure 8(B,C) we only included $\Delta T_{\text{max}}$ of the experimentally applied pulses with MEDIAN($\Delta T_{\text{max}}$) = 9.49$^\circ$C and the range 1 $\leq$ $\Delta T_{\text{max}}$ [$^\circ$C] $\leq$ 39 for a median duration of 80 s (1.33 min) with (0.2 $\leq$ Total treatment time [min] $\leq$ 9), implying the presence of both mild-hyperthermic, and thermally ablative effects in the IRE-TR, which accords the findings in [13–15]. Please note that the duration of these effects depends on the selected experiment. For example, according to Figure 11 the duration of mild hyperthermia during IRE has a median of 15 s with the range 0 $\leq$ $\Delta t$ [s] $\leq$ 100, while the duration of thermal ablation has a median of 0 s with the range 0 $\leq$ $\Delta t$ [s] $\leq$ 133.3. These durations were calculated at a single point where $\Delta T_{\text{max}}$ was obtained (only during the temperature increase). Accordingly, the durations of both effects can be longer when considering the decay of the temperature post-IRE treatment.

Nevertheless, $\Delta T_{\text{max}}$ does not specify the size of these effects. Accordingly, in Figure 9 we plotted the ratios of the mild-hyperthermic and thermally ablative effects with respect to IRE-TR as a function of the IRE-TR. As expected, the increase of the IRE-TR results in the increase of the $\Delta T_{\text{max}}$, which implies that the increase of IRE-TR raises the $\Delta T_{\text{max}}$ and therefore, enlarges thermally ablative effect.

### 4.2. Energy density

Concerning the energy density of the experimentally applied pulses in Figure 10(A) and considering Equation 23 one would expect to find a positive linear relationship between the energy density and $\Delta T_{\text{max}}$. Indeed, by using the linear regression analysis we do observe that an increase in the energy density results in an increase in $\Delta T_{\text{max}}$. However, the data points do not show a clear correlation, because of the many different system configurations used as mentioned above. This also applies to Figure 10(B), which shows the relationship between energy density and the ratios $R_{3\Delta T_{13}}$ and $R_{3\Delta T_{13}}$.

### 4.3. Selection of model parameters

According to Figure 7(A–E), the selection of tissue properties varies between studies, even within the same tissue category. One reason for these variations is the inclusion of subcategories within the main categories. For example, ‘Brain’ can represent gray and white matter, ‘Blood vessel’ represents artery, blood and blood vessel, and ‘Muscle’ can...
represent longitudinal and transversal muscles in breast, prostate, and small intestine. Other reasons that can explain the variations are: (1) the anisotropicity and the heterogeneity of a tissue sample, (2) the location of the selected sample in an organ, (3) the animal species, and (4) the applied method to determine a property of a tissue type.

Due to the similarities of the values of the thermal properties in Figure 7(B–D), one would expect the perfusion term \( w_b \) to have a great influence on \( \Delta T_{\text{max}} \). Nevertheless, no correlation was found between these two parameters due to variation in the selection of other model properties, such as the boundary conditions and electrode type.

Finally, Figure 7(F) shows variation in the selection or calculation of \( E_{\text{IRE(13)}} \) between studies, even within the same tissue categories. Reasons that explain these variations are: (1) the application of different pulse parameters, (2) the choice of static or dynamic electrical conductivity, (3) the choice between the modeling of homogeneous or heterogeneous tissue type, (4) the applied electrode configuration; in comparison to plate electrodes, we expect larger mild-hyperthermic region in case of needle electrodes due to higher electric field density, with as a result a larger treated region and lower \( E_{\text{IRE(13)}} \).

Studies including the perfusion term in the bioheat transfer models applied constant blood perfusion. However, the blood perfusion is dynamic and depends on the magnitude and the duration of the thermal exposure, the tissue type, the size of the cancerous tissue, and whether a small or large

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**Figure 10.** (A) Linear regression plot of \( \Delta T_{\text{max}} \) data points as function of the energy density. (B) Linear regression plots of \( R_{\Delta T_{13}} \) and \( R_{\Delta T_{13}} \) data points as function of the energy density. Please note the change in X-axis in (B) in comparison to (A).
animal model, or human model was used, as was shown in a review paper performed by Rossmann and Haemmerich [94] that summarized on the temperature and tissue-dependent thermal properties, including the blood perfusion, of several biological tissues at mild-hyperthermic and ablative temperatures. Based on figure 6 of [94], and since the medians of both simulation time and temperature increase of the included studies approximated 1.3 min (80 s) and 10°C (47°C relative to the assumed body temperature), we believe that temperature-dependent perfusion should be considered at T ≥ 43°C for short total treatment time and at T ≥ 41°C for long total treatment time. The latter would be preferred in treatment planning due to the presence of several needle pairs (more than 3 needle pairs in general; each pair with an approximation of 90 pulses and 10 test pulses) that may allow the extension of the total treatment time for more than 10 min. Nevertheless, the data about IRE-targeted organs are still limited. Therefore, further research should be performed to determine the influence of the magnitude and duration of thermal exposure.

4.4. Estimation of time durations of thermal effects

According to the extracted data and our simplified analysis, the mild-hyperthermic effect has a median time duration of 15 s, while thermally ablative effect has a median time duration of 0 s (ranges from 0 to ~130 s) during the IRE treatment at the point with maximal temperature increase. Assuming a total treatment time of 80 s, this means that the combined medians of the time duration of the thermal effects would occur for approximately 20% of the treatment time (mild-hyperthermic effect for ~20% and thermally ablative effect for 0%). However, these estimations relate to the duration during the IRE treatment itself and both effects will also last post IRE treatment. As a result, larger medians could be estimated for the total time durations. It is important to notice that the durations were calculated at the spatial point with ΔTmax. Since mild-hyperthermic temperatures spatially dominates the TA temperatures, the overall time duration of the mild-hyperthermic temperature, especially around the zones with T ≥ 50°C (e.g. zones neighboring the electrodes), is longer than the time duration of TA effects. Furthermore, the studies included in the analysis used two electrodes in general. In the clinic, the number of electrode pairs is generally higher and could increase above 6 (more than 4 electrodes could be used), possibly resulting in further increase of ΔTmax and the mild-hyperthermic and ablative time durations. In particular, this applies for mild-hyperthermic effect, since an adequate order of the electrode pairs could avoid the extension of TA effects as was shown by O’Brien et al. [95]. O’Brien et al. showed in an ex vivo study using a perfused porcine liver model that cycled pulsing schemes increase the treatment zone size and maintain a low tissue temperature compared to conventional pulsing schemes.

Our analysis can include estimation errors, since (1) the temperature profile has a temporal exponential profile if our simplified assumptions were not taken into consideration, and (2) only some of the simplified assumptions were assumed by the included studies. However, by comparing the extracted time durations with the calculated ones, a deviation of ≤22% was found. Finally, one must note that the included studies in which multiple electrode pairs were used did not consider the delays between the pulse sequences. In the clinic, delays are applied between the pulse sequences of different electrode pairs, both to recharge the pulse generator and to reduce the produced ΔTmax.
taking these delays into consideration, one would expect a reduction in thermal effects. Nevertheless, only a slight reduction of the combined median time duration of the thermal effects is expected since only two out of the 12 included studies applied IRE with 4 electrodes which involve activating four different electrode pairs during IRE [33,41].

4.5. Inclusion of vascular structures

The majority of the included studies did not incorporate vascular structures with blood vessels in the models [3,24,26,39–42]. Due to the high electrical conductivity of blood, the inclusion of vascular structures in or in close proximity of the treatment volume can result in distortion of the distribution of the electric field strength inside the treatment volume. Consequently, neighboring areas with relatively high or low electric field strength can emerge, resulting in undesirable overtreatment or undertreatment of those areas with possible clinical complications or treatment failure as a consequence. Several experimental and in silico studies showed that the distortion of the distribution of the electric field strength resulted in reduction of the electric field strength in areas inside the treated zone adjacent to the vascular structure; the ‘electric field sink’ effect [54,62,71,96]. For example, Goldberg et al. experimentally showed undertreated zones adjacent to vascular structures in the liver of rats after application of IRE between two electrode plates [54]. We therefore recommend the inclusion of vascular structures in treatment planning to avoid possible undesired poor quality treatments and to accurately predict the local electric field and temperature distribution.

Furthermore, large blood vessels can yield a substantial cooling effect in the ablated region, similar as for RFA and MWA thermal ablation, which can result in a local temperature decrease of more than 20 °C [97]. Several experimental and in silico studies showed a much lower temperature increase in the vicinity of a large blood vessel during RFA or MWA, dependent on the orientation of the vessel and its distance to the applicator [97–100]. This demonstrates the relevance of accounting for large blood vessels also in IRE models, since a local cooling effect may significantly affect the produced thermal increase in the close vicinity of large vessels, which may result locally in only an irreversible permeabilization effect. Van Gemert et al. demonstrated in a 1D analytical IRE model that a large blood vessel at ~5 mm distance from a needle can produce a noticeable cooling effect up to a few degrees [64]. A simplified model not including large vessels would be sufficient when for example evaluating maximum temperature levels achievable during IRE. However, when accurate predictions of the local temperature distribution are required, the effect of nearby large blood vessels should be accounted for in the models.

4.6. Challenges of simulations

The results presented in this review are based on calculations extracted from several studies with various simulated setups. Specifically, the extracted data may include errors, due to e.g. inaccurate modeling of the media (homogeneous instead of heterogeneous), inaccurate modeling of the simulated setup, inaccurate choice of boundary conditions, inaccurate assignment/modeling of electrical and thermal properties, and inaccurate choice of pulse characteristics (e.g. not modeling the delays within a single pulse sequence and between separate pulse sequences of different electrode pairs will result in overestimations of temperature). Another example is the modeling of $\sigma(T)$, in which $\sigma$ was modeled as a function of temperature (see Supplementary Appendix 4 for more details). Specifically, the electrical conductivity can increase by 1–3% per 1 °C [50]. However, the increase of $\sigma$ stops after a certain temperature due to the vaporization process of water in the medium. Therefore, a certain threshold should be included in $\sigma(T)$ and $\sigma(E, T)$ to avoid $\sigma$ to further increase after a certain temperature. This temperature is typically 100 °C, which was applied by several RFA studies [101,102]. Despite these uncertainties in the modeling studies, the data obtained in this review still provides useful insight in the possible thermal elevation by IRE.

4.7. Take-home message

This review demonstrates the likelihood of direct thermal ablation in comparatively small regions and mild-hyperthermic effects in a large part of the IRE-TR, predicting the significant presence of hyperthermia in IRE. Even though the thermally ablated regions were relatively small, care must be taken for large temperature increases, especially in case of adjacent critical structures. This review focused on studies that performed numerical calculations, which are very useful to obtain insight in the general impact of various pulse parameters on the effect of IRE. Numerical calculations typically do not model nanopore effects and assume a simplified anatomy, not accounting for variations in tissue properties. Nevertheless, results clearly indicate that IRE can have significant thermal effects. Therefore, further in vivo research should investigate thermal effects of IRE to determine the relative size of the irreversible permeabilized region in which mild-hyperthermic and thermally ablative temperatures are achieved and significantly contribute to tumor ablation. These insights will help to optimize clinical protocols of IRE.

The expression ‘non-thermal IRE’ is generally used, (1) because IRE is a technique based on the creation of nanopores in the cell membrane, and (2) to distinguish IRE from thermal ablation techniques such as RFA and MWA. However, due to the significant presence of thermal effects (including 40 ≤ $T$ [°C] < 50), and since literature data demonstrate that thermal effects clearly do contribute to the tumor ablation, one might consider avoiding the expression ‘non-thermal’ and start focusing on researching the mild-hyperthermic contribution to further optimize IRE-treatment.

Disclosure statement

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