Recei...
Additionally, to better understand electric field threshold changes with respect to pulse number, the IRE response of pancreatic adenocarcinoma cells in tumor mimics was characterized.

2 METHODS

2.1 Computational model

All relevant structures were reconstructed from axial, sagittal, and coronal planes using Tri-phase thin (0.75 mm) cut CT Images. The segmentation and reconstruction of the relevant anatomy was acquired from Analogic Corp. (Peabody, MA), in which proprietary software was used. The geometry was later meshed using 3-matic software (Materialise, Leuven, Belgium).

Electrode geometries were created using FreeCAD and imported into 3-matic for electrode placement and meshing as stereolithography (.stl) files. During electrode placement, most of the patient anatomy was kept as a reference. Once the electrodes were placed, all structures that did not significantly affect electric field distribution during treatment delivery were excluded.

2.1.1 Electric field distribution and conductivity considerations

The 3D reconstruction was imported into a finite element analysis (FEA) software package, COMSOL Multiphysics® (COMSOL, Stockholm, Sweden) to simulate the IRE protocol. The electric field distribution was found by solving the following differential equation:

$$\nabla \cdot (\sigma \nabla \varphi) = 0$$

where $$\sigma_d$$ is the electrical conductivity of the tissue, which is dynamic and dependent of the electric field magnitude, and $$\varphi$$ is the applied voltage. The following function was used to describe behavior of $$\sigma_d$$ during IRE:

$$\sigma_d = \sigma_0 + \sigma_\Delta e^{-e^{(E - E_{th})}}$$

where $$\sigma_0$$ is the pre-IRE conductivity, $$b$$ is a constant defining the rate at which $$\sigma_d$$ changes (set to 0.2), E is the electric field magnitude, and $$E_{th}$$ is the reversible electroporation threshold (400 V/cm). The conductivity range, $$\sigma_\Delta$$ (Table 1) was determined from previous observations in kidney with similar pulse protocols where amperage values were comparable to those observed in pancreas. Boundary conditions for the electrode-tissue interface were set to $$\varphi = V_0$$ on one electrode and $$\varphi = 0$$ at the counter electrode boundary. All other boundaries were assumed electrically insulative.

2.1.2 IRE thresholds

Electric field thresholds for pancreatic cell death post-delivery of IRE pulses have not been characterized in vivo. As a surrogate of pancreatic adenocarcinoma tissue and a guide toward understanding the effects of sequentially increasing pulse delivery, electric field thresholds for cell death were characterized on PANC1 tumor mimics ($$n = 3$$) as described in other studies.

2.1.3 Thermal considerations

Joule heating effects associated with IRE pulses were calculated by solving the following modified Penne's bioheat equation:

$$\nabla \cdot (k \nabla T) + \frac{\sigma |\nabla \varphi|^2}{\tau} + Q_{met} + w_b \rho_b c_b (T_b - T) = \rho c_p \frac{\partial T}{\partial t}$$

where $$Q_{met}$$ is the metabolic heat source, $$\rho$$ is the density, $$c_p$$ is the heat capacity, and $$\tau$$ is the relaxation time.

<table>
<thead>
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<th>Material</th>
<th>Property</th>
<th>Symbol</th>
<th>Value</th>
<th>Ref.</th>
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</table>
where $T$ is the temperature (initial value set to 37°C), $w_b$, the blood perfusion rate (from adrenal gland), and $T_b$ is temperature of blood (set to 37°C). The Joule heating term, $\sigma\nabla \varphi^2$ was altered by a factor directly related to the ratio of pulse duration $\tau$ and pulse interval $t$ which is used to reduce computational time. Tumor tissue was assigned the properties of healthy pancreas since this data are not readily available. Other variable nomenclature and values used in this model can be found in Table 1.

2.1.4 Protocol verification

Tumor geometry was set as a variable and assigned its expression as the normal of the electric field. Once a solution was reached the ratio in between total tumor volume and the volume of tumor tissue exposed to a marginal electric field value (e.g., 500 V/cm) was calculated. Protocols were modified until this ratio approached 1.

2.2 Treatment delivery and resistance monitoring

Three patients received IRE treatments for LAPC as described in. Open laparotomy allows for needle placement in caudal-cranial fashion, which has been found to be safer and more effective than percutaneous approach. Our group developed a resistance monitoring system that can interact with the commercially available IRE device, the NanoKnife (Angiodynamics, Queensbury, NY). The high-voltage signal delivered to the tissue was passively measured through a custom made device that connects to both the IRE electrodes and a portable computer. The portable computer displayed a user interface that allowed physicians to track changes in resistance real-time.

2.3 Postoperative management

Postoperative management of patients treated with IRE followed guidelines for any type of pancreatic resection. More details regarding follow-up of patients are provided by Martin et al.

2.4 Statistical analysis

Data were analyzed using R statistical software (R Foundation for Statistical Computing, Vienna, Austria, 2015). A t-statistic was used to test the difference between means for pulse delivery with and without the implementation of the resistance measurement system. Resistance data were expressed as means ± standard deviation of samples. The approach was implemented for IRE thresholds for cell death in collagen I matrices. For all tests, a one-tailed $P < 0.01$ was indicative of statistical significance.

3 RESULTS

Following segmentation and meshing of tumor, pancreas, superior mesenteric vein, and superior mesenteric artery a patient-specific geometry was obtained (Fig. 1). Figure 2a shows the fully reconstructed anatomy, which was optimized for reduction of
computational time in Fig. 2b. The optimized mesh in Fig. 2c resulted 2-3X faster computational times (~1 h) than the initial geometry.

IRE electric field thresholds for pancreatic cancer cells were assessed using a three-dimensional tumor mimic as described in Arena et al. A representative example of a treated tumor mimic is presented in Fig. 3a. Ablations were not apparent when the treatment consisted of only five pulses. Regions of dead cells were observed for treatments consisting of 10-200 pulses with electric field thresholds decreasing from approximately 900-500 V/cm over this range (Fig. 3). Changes in lethal thresholds with respect to pulse delivery were visualized using the reconstructed patient geometry in Fig. 2. When examining thresholds found in the tumor mimics, the solution for a single electrode-pair predicts a clinical ablation of 1.36 cm³ for 10 pulses, 3.44 cm³ for 100 pulses, and 3.55 cm³ for 200 pulses. The temperature profile can be observed in Fig. 4. The solution of the bioheat model for the proposed example resulted in a maximum temperature of 45.2°C.

The temperature at the midpoint is not sustained above 40°C for more than 1 min after the conclusion of a 90 pulse procedure.

Figure 5 presents a case in which the patient received 100 fewer pulses through one probe pair than originally planned. This decrease in applied pulses was based on reaching a decline in tissue resistance...
of 28 Ω, which could not have been achieved without the implementation of a real-time resistance measurement system. Overall, when delivering sets of 100 pulses, a more pronounced change in resistance between electrode-pairs was observed during the first set of pulse delivery. For the cases presented in Fig. 6, an estimate of 777 pulses were not delivered by following 28 Ω clinical endpoint, which reduced the ablation time by approximately 29 min. Statistical analysis of pulse delivery showed that by implementing the proposed resistance measurement system 210 ± 26.1 less pulses could be delivered per electrode-pair during IRE ablations of pancreatic tissue (P < 0.01).

**FIGURE 5** Changes in resistance during IRE treatment of locally advanced pancreatic adenocarcinoma. All probe pairs received an initial 200 pulses at 1500 V/cm voltage-distance ratio. Surgeon proceeded to reposition all electrodes by 1.2 cm in the axis of insertion towards the surface of the tissue. For a) probe pair 1-3 treatment was stopped at 300 pulses based on resistance readings while b) probe pairs 1-2 received a total 400 pulses.

**FIGURE 6** MR images (two axials, one sagittal) of LAPC tumor before and after IRE using resistance monitoring system for 4-electrode array in a 76-year-old male patient. a-c) Pre-IRE sequential MRIs, demonstrates abutment of SMA and PV/SMV occlusion, and SMV reconstitution at end (arrows). d-f) Three months post-IRE, stable complete IRE with no evidence of worsening vascular arterial impingement, and no evidence of pancreatic mass enhancement (arrows).

**4 | DISCUSSION**

Collagen type I matrices have been shown to enhance the morphology and physiological-like behavior of cells, such as angiogenic factor secretion and drug responsiveness. This platform has proven helpful for testing different molecular and physics-based therapies, which includes IRE. Figure 3 provides evidence that the change in IRE threshold with respect to pulse number reaches clinical relevance (~500 V/cm) in pancreatic tumor mimics at 100 pulses. Unlike intraoperative resistance measurements where resistance changed significantly between 100 and 200 pulses, IRE thresholds in tumor...
mimics were stable between 100 and 200 pulses. It is possible that ablations within the target volume do not fully develop in the first 100 pulses for in vivo procedures.28

The above-described procedure and parameters also provide a tool that allows physicians to avoid substantial thermal insult to the tissue of interest. The proposed Joule heating model agrees well with experimentally recorded data by Dunki-Jacobs et al where temperature measurements were recorded at several points in the ablation region as IRE pulses (1500 V/cm voltage-to-distance ratio and 90 100 μs pulses) were delivered to porcine pancreas.29 In Fig. 4, though the model’s separation across the two electrodes varies by 3 mm, the change in maximum temperature at the midpoint between the electrode-pairs used is comparable (10.3°C vs. 8.2°C for the experimental and the proposed model, respectively). This difference could be attributed to longer electrode exposures used in the experimental procedure which should result in higher amperage levels.

Real-time resistance measurement during IRE procedures is currently the only method available for feedback mainly because it can be incorporated seamlessly to the surgical workflow. The proposed measurement system can save a significant amount of OR time by avoiding unnecessary pulses while ensuring that the therapy reaches a clinical endpoint, which has been set at a delta value of 28 Ω. Images of three patients receiving ablations under real-time measurement system showed complete tumor ablation while avoiding unnecessary energy delivery (Figs. 5 and 6). For therapies incorporating four electrodes a potential 800 or more pulses could be avoided. As observed in Fig. 4, electrode-pair 1-2 exhibits a higher change in resistance, which can be attributed to this pair being the first set to receive IRE pulses. Additionally, the exponential decay in resistance observed in probe pair 1-2 agrees with the trend for IRE thresholds found in tumor mimics.

While computational models showed good agreement with experimental data, it is important that future work characterizes the bulk electrical response of malignant and healthy pancreatic tissue to IRE. A study similar to those described in Neal et al9 and Bonakdar et al15 in which biopsied tissue samples undergo an impedance analysis before and after ablation would add significant value to the proposed pre-treatment planning protocol. When IRE pulses are delivered without adequate pre-treatment planning significant temperature increases near the electrodes may compromise the quality of electrical resistance measurements. Moving forward, the proposed real-time measurement system could be implemented on an individual basis through collaborations between clinicians and engineers, or at a broader scale by modifying the software of the commercially available device to allow real-time data display.

5 | CONCLUSION

The established treatment planning methodology for IRE procedures in pancreas proved a helpful tool for optimization of ablation parameters and has potential to improve clinical outcomes on a patient-specific basis. Lastly, a method for confirming complete ablation per electrode-pair through intraoperative monitoring of tissue resistance proved useful by significantly reducing ablation times and risk of thermal effects. The proposed physics-based treatment plan through FEA coupled with monitoring of resistance changes allows for optimization of probe location, output voltage, and number of pulses while avoiding significant thermal damage.

DISCLOSURES AND FUNDING SOURCES

This study was performed in compliance with HIPAA and hospital IRB approval. The corresponding author is a paid consultant for AngloDynamics, Inc. and other authors have issued patents in IRE technology.

REFERENCES
