Microwave and Radiofrequency Ablation: A Comparative Study between Technologies in Ex Vivo Tissues

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Abstract: In this paper, we report on the use of a purpose-built hybrid solid-state microwave and radiofrequency generator operating at frequencies of 2.45 GHz and/or 480 kHz for cancer ablation in various tissues. The hybrid generator was tested ex vivo on chicken breast and bovine liver and has demonstrated that the high accuracy of the power delivered to the sample can be achieved by controlling the emitted power versus the temperature profile of the treated sample. In particular, the hybrid generator incorporates control systems based on impedance or reflected power measurements that allow controlled ablation without causing unwanted carbonization and without including areas where tissue damage is not desired. The results of the ex vivo tests showed that radiofrequency ablation (RFA) could be effective for performing controlled ablations with minimally invasive probes, such as cardiac pathologies, small lesions, and tissues with particular composition, while microwave ablation (MWA) could be optimal for performing large ablations in highly vascularized tissues, such as liver cancer, where it is necessary to achieve higher temperatures.

Keywords: microwaves; radiofrequency; medical applications; medical equipment; solid-state; tissue ablation; cancer; temperature control; ex vivo

1. Introduction

The use of microwaves and radio waves has a rich history that has transformed many aspects of our daily lives. From the first radio signal transmissions in the late 19th century, pioneered by scientists like Heinrich Hertz and Guglielmo Marconi, these signals enabled wireless communication, revolutionizing information and communication systems. Later, this technology turned out to be crucial to the discovery of microwaves in the 1930s. This form of electromagnetic radiation, with wavelengths shorter than radio waves, opened up new possibilities during World War II, leading to the development of radar, which was vital for military defense [1]. In the post-war period, the technology was then adapted for civilian applications, giving birth to the microwave oven, commercially introduced in the 1960s, which transformed how we cook and heat food [2]. Simultaneously, microwaves became essential for telecommunications, including satellite systems and terrestrial microwave links.

Today, both microwaves and radio waves are integral to modern technologies, from the everyday use of cell phones and Wi-Fi to medicine and advanced scientific research. The energy carried by these electromagnetic (EM) waves is utilized for heating up different materials in liquid and solid phases and to bring gases to the plasma state, a highly reactive state of matter applicable in various fields, ranging from semiconductor production [3] to hydrogen generation for clean energy [4].
1.1. Radiofrequency and Microwave Techniques in Medical Applications

Interactions of the EM field with the human body have been used in medicine, i.e., cardiology, oncology, physiotherapy, and urology since the late 1970s. Currently, EM fields are frequently used in a few well-established medical procedures. The medical applications of radiofrequency (RF) and microwave (MW) radiations can be divided into three main groups, i.e., diagnostics, medical equipment, and therapy.

A good example of the use of RF in diagnostic imaging is magnetic resonance imaging (MRI) [5]. RF generators can also be part of more complex medical devices, such as particle accelerator-based imaging and therapy devices [6,7], or can be used to assist in surgical procedures, such as in electrosurgical and radiosurgical devices. In the therapeutic field, some of the most common applications in recent years have been radiofrequency ablation (RFA) and microwave ablation (MWA) [8].

MWA and RFA thermal ablation use a local increase in temperature to induce the denaturation of cellular membrane proteins and kill cells. These procedures can be used not only for cancer treatment [9], but also for pain management [10], physiotherapy [11], cardiac arrhythmias [12], benign tumors [13], and other applications [14,15]. There are several differences between the two technologies, which do not mean that one is always more effective than the other [16]. RFA is a well-established treatment with a long track record of success since the 1990s [17], whereas MWA has only been used since the 2000s [18] and on a larger scale in the last decade. Despite this, MWA is a faster technique that can be used more efficiently to treat larger tumors [19]. Due to the lack of a standard procedure [20] and the continuous improvements in technology and applications [21], the skill and experience of the physician play a fundamental role in the choice of procedure type, although this means a greater burden of responsibility and less repeatability of results, especially today, as medical device manufacturers strive for higher performance, less invasive methods at competitive costs, and new technologies and production methods. Minimally invasive thermal ablation is now widely used for the treatment of focal tumors of the liver, lung, kidney, and bone that are considered early-stage or refractory to conventional therapies such as surgical resection. Several modalities have been investigated, but RFA and MWA remain the most widely used overall [22–26].

RF and MW are a part of the EM spectrum characterized by frequency, wavelength, and energy. The different RF and MW frequency bands that have been allocated for medical use and their main medical applications are shown in Table 1.

<table>
<thead>
<tr>
<th>Frequency Band</th>
<th>Medical Applications</th>
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<tr>
<td>3–30 kHz</td>
<td>Electrotherapy, nerve stimulation</td>
</tr>
<tr>
<td>100–300 kHz</td>
<td>Shortwave therapy, electrotherapy</td>
</tr>
<tr>
<td>300–3 MHz</td>
<td>Shortwave diathermy, therapeutic tissue heating</td>
</tr>
<tr>
<td>3–10 MHz</td>
<td>Shortwave diathermy, capacitive diathermy</td>
</tr>
<tr>
<td>13.553–13.567 MHz</td>
<td>Shortwave diathermy, therapeutic tissue heating</td>
</tr>
<tr>
<td>26.957–27.283 MHz</td>
<td>Diathermy, therapeutic tissue heating</td>
</tr>
<tr>
<td>40.66–40.70 MHz</td>
<td>Short-range communication devices for patient monitoring</td>
</tr>
<tr>
<td>433.05–434.79 MHz</td>
<td>Telemetry, monitoring devices, therapeutic tissue heating</td>
</tr>
<tr>
<td>902–928 MHz</td>
<td>Medical telemetry, remote monitoring, therapeutic tissue heating</td>
</tr>
<tr>
<td>2400–2500 MHz</td>
<td>Glucose monitoring, drug delivery, therapeutic tissue heating</td>
</tr>
<tr>
<td>5725–5875 MHz</td>
<td>Short-range communications, monitoring</td>
</tr>
<tr>
<td>24–24.25 GHz</td>
<td>Respiratory and heart rate monitoring</td>
</tr>
</tbody>
</table>
These frequencies are chosen for their ability to penetrate biological tissues and their effectiveness in generating heat or stimulating nerves and muscles, providing therapeutic benefits without causing significant tissue damage. In addition, specific frequencies such as 13.56 MHz, 27.12 MHz, 433 MHz, 915 MHz, and 2.45 GHz are also used for tissue ablation, a procedure in which targeted heat is used to remove or destroy abnormal tissue. Some applications at 5.8 GHz are currently being tested.

The wide range of applications using RFA and MWA equipment opens up new opportunities for research in general, and medical research in particular. One of the focal points in the development and dissemination of these technologies is the introduction of RF and MW solid-state generators. Compared to magnetron-based generators, solid-state MW generators use semiconductor materials, i.e., transistors such as LDMOS (laterally diffused metal oxide semiconductor) and GaN (gallium nitride), to generate EM waves. Solid-state generators can deliver power in continuous wave (CW) or pulsed form, and are capable of producing an excellent frequency spectrum over the entire power range from the very first watts. They are significantly smaller than magnetron-based generators, are more reliable, have a longer operating life, are easier to handle, and offer a higher level of control. Other advantages include frequency and phase variability and control, and better compatibility with other electronic circuits.

1.2. Interactions of Electromagnetic Radiation with Biological Tissue in Thermal Heating

RF and MW fields can penetrate and propagate through the human body. As these waves propagate through biological tissues, their energy is gradually absorbed and converted into heat, increasing the temperature of the exposed area. The mechanisms of MW and RF heating and thus ablation are based on hyperthermia, i.e., dielectric heating effects designed to cause coagulative necrosis within the targeted tissue. The mechanisms of dielectric heating and its relationship to ablation have been addressed in numerous medical publications [23,28] and the effects are generally attributed to the rapid and selective heating of water—a polar molecule—present in tissues. However, it should be emphasized that dielectric heating is usually referred to as the rapid and selective heating of a material by EM fields, and that the EM waves are synchronized oscillations of electric and magnetic fields. For example, the electric field of MW at a frequency of 2450 MHz alternates at a rate of $2.45 \times 10^9$ oscillations/s. Therefore, dielectric heating can involve polar molecules (such as water), semiconductors or even conducting metallic or non-metallic materials. It is also important to note that RF and MW are non-ionizing radiations, i.e., their energy is not sufficient to ionize atoms or molecules; however, dielectric heating applied to medical applications can offer advantages in terms of speed and control of heating.

Both RF and MW are techniques that allow a portion of the material/load (e.g., body tissue) to be heated volumetrically due to the interaction of the EM field with the load. The extent of the heated area is strictly dependent on:

- The nature of the load and its temperature (also influenced by the blood flow);
- The frequency of the EM wave, its applied power density (power per unit volume dissipated in the load), and its penetration depth, i.e., the distance at which microwave power is reduced to $1/e$ ($e = 2.718$) = 0.368 or ~37% from the strength at the point of entry and can be a limiting factor in application if the mechanism involved is not properly understood.

The interaction of a load with an alternating electric field is usually expressed in terms of charge redistribution or polarization, which can be:

- Electronic—electrons which have low mass and small inertia and can easily follow the alternations of the electric field, are shifted relative to positive nuclei, resulting in a non-zero dipole moment;
- Dipolar—the alternating electric field tends to align the dipoles in the load parallel to the field and depending on the mass of the dipolar molecule and the frequency of the wave, a phase difference is created between the orientation of the electric field and the molecules leading to dissipative interactions and heat generation;
• Ionic—positively and negatively charged ions are shifted out of their barycenter, leading to vibrations of atoms or ions;
• Interfacial polarization—displacement of free charges accumulated at the interface located within the load under the influence of the electric field.

These interactions are dissipative and lead to heat generation and may be accompanied by magnetic losses, especially in conductive loads such as those containing metals (hysteresis, eddy currents, domain wall, and electron spin resonance) [29].

In dielectric heating applications, it is necessary to quantify the contribution of all these interactions. This is usually expressed in terms of the complex relative permittivity, a measure of how the alternating electric field interacts with the load. The relative permittivity depends on the frequency of the wave, the temperature, and the composition of the load, as shown in Tables 2 and 3 adapted from [30], which means that MWA and RFA techniques must be adapted in relation to these parameters.

Table 2. Dielectric constant ($\varepsilon'$) and conductivity of body tissues vs. electromagnetic frequency [30].

<table>
<thead>
<tr>
<th>Tissue Type</th>
<th>Density (app.) g/cm$^3$</th>
<th>13.56 MHz</th>
<th>915 MHz</th>
<th>2450 MHz</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$\varepsilon'$</td>
<td>Conductivity S/m</td>
<td>Permittivity</td>
<td>Conductivity S/m</td>
</tr>
<tr>
<td>Avg. brain</td>
<td>1030.0</td>
<td>208.231766</td>
<td>0.251501</td>
<td>45.745209</td>
</tr>
<tr>
<td>Avg. skull</td>
<td>1850.0</td>
<td>44.938148</td>
<td>0.086985</td>
<td>16.597830</td>
</tr>
<tr>
<td>Avg. muscle</td>
<td>1040.0</td>
<td>132.074387</td>
<td>0.655560</td>
<td>55.920837</td>
</tr>
</tbody>
</table>

N.B. Water $\varepsilon'$~80; conductivity between 0–1.5 S/m; contribution to ionic conduction dominates at frequencies <600 MHz.

Table 3. Dielectric constant ($\varepsilon'$) and loss tangent (tan $\delta$) vs. electromagnetic frequency for different organs/body tissues [30].

<table>
<thead>
<tr>
<th>Tissue/Organ Type</th>
<th>13.56 MHz</th>
<th>915 MHz</th>
<th>2450 MHz</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$\varepsilon'$</td>
<td>tan $\delta$</td>
<td>$\varepsilon'$</td>
</tr>
<tr>
<td>Blood</td>
<td>210.676758</td>
<td>1.117084</td>
<td>61.313965</td>
</tr>
<tr>
<td>Bone cancellous</td>
<td>59.301670</td>
<td>0.128449</td>
<td>20.755970</td>
</tr>
<tr>
<td>Kidney</td>
<td>297.137695</td>
<td>0.542046</td>
<td>58.557178</td>
</tr>
<tr>
<td>Liver</td>
<td>181.262329</td>
<td>0.335616</td>
<td>46.763901</td>
</tr>
<tr>
<td>Lung inflated</td>
<td>95.402214</td>
<td>0.236064</td>
<td>21.971907</td>
</tr>
<tr>
<td>Lung deflated</td>
<td>148.697876</td>
<td>0.451530</td>
<td>51.372128</td>
</tr>
</tbody>
</table>

N.B. Water at 20°C: 2450 MHz–$\varepsilon'$~80, $\delta$~0.157; 915 MHz–$\varepsilon'$~80, $\delta$~0.045; <100 MHz–$\varepsilon'$~80, $\delta$~0.005; $\varepsilon'$ represents the capacity of a load to store energy; $\varepsilon''$ is the loss of energy by relaxation (of importance at 915 MHz and 2.45 GHz); tan $\delta = \varepsilon''/\varepsilon'$. The higher its value the more absorption of the electromagnetic energy by the load.

RFA devices create a simple electrical circuit through the body, using an oscillating electrical current to create resistive heating within the tissue surrounding an interstitial electrode. Because tissues are poor conductors of electricity, the current flowing through the tissue causes ionic movement and the generation of frictional heat. Therefore, the areas closest to the electrode experience the highest current and therefore a greater increase in temperature. Tissues further away from the electrode are heated primarily by conduction. The circuit is completed by a dispersive electrode, typically placed on the patient’s skin in a monopolar system, or by a second interstitial electrode in bipolar RFA [31,32].

Unlike RFA, MW energy is not an electric current but a propagating EM field that interacts with molecules via the mechanisms described above. Although the study of the effect of MW frequency on tissue interaction is beyond the scope of this article, it is worth mentioning that the effect of changing the microwave frequency, 915 MHz vs. 2.45 GHz, has been addressed in several studies [33–35]. A review of the literature shows that both 915 MHz and 2.45 GHz systems are capable of producing large, clinically useful ablation zones, but that not all systems are equal, which is supported by differences in dielectric
parameters as a function of frequency, as shown in Table 3. In addition to the lack of experience associated with new techniques or devices, which initially affects short-term results, the design, power settings, experimental model, and data analysis techniques should be carefully interpreted when evaluating an MWA system for clinical use.

2. Results

The results in Table 4 show higher Roundness Index (see Section 3.5.1) and size with MWA. In general, all sizes of ablated tissue were smaller with RFA due to the occlusive effect of the dried tissue with its high impedance at RF. The dimensional difference can also be observed between the results obtained on chicken breast and bovine liver—Figure 1, due to the different electrical properties and less homogeneous composition of bovine liver, which has a denser vascularization that limits heat propagation.

Table 4. MWA and RFA—dependence of ablation dimensions and roundness index vs. MW and RF power. Sizes and roundness index presented as means ± standard deviation (each row is the result of 15 tests).

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<tbody>
<tr>
<td>50</td>
<td>20.1 ± 2.2</td>
<td>18.5 ± 2.5</td>
<td>0.90 ± 0.02</td>
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<tr>
<td>100</td>
<td>33.2 ± 3.0</td>
<td>30.2 ± 3.2</td>
<td>0.91 ± 0.01</td>
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<tr>
<td>150</td>
<td>37.1 ± 4.1</td>
<td>32.4 ± 3.5</td>
<td>0.86 ± 0.01</td>
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<tr>
<td>200</td>
<td>48.3 ± 4.0</td>
<td>43.3 ± 4.1</td>
<td>0.89 ± 0.01</td>
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<tr>
<td>250</td>
<td>50.2 ± 4.2</td>
<td>45.2 ± 3.3</td>
<td>0.90 ± 0.01</td>
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<tr>
<td>50</td>
<td>18.4 ± 1.8</td>
<td>15.1 ± 2.3</td>
<td>0.83 ± 0.03</td>
<td></td>
</tr>
<tr>
<td>100</td>
<td>32.2 ± 1.9</td>
<td>25.2 ± 2.2</td>
<td>0.78 ± 0.01</td>
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<tr>
<td>150</td>
<td>35.3 ± 2.8</td>
<td>28.2 ± 3.2</td>
<td>0.80 ± 0.02</td>
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<tr>
<td>200</td>
<td>40.1 ± 3.7</td>
<td>33.1 ± 3.5</td>
<td>0.83 ± 0.01</td>
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<tr>
<td>250</td>
<td>42.2 ± 4.3</td>
<td>35.3 ± 4</td>
<td>0.83 ± 0.01</td>
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<tr>
<td>10</td>
<td>22.2 ± 1.3</td>
<td>12.5 ± 1.7</td>
<td>0.55 ± 0.01</td>
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<tr>
<td>20</td>
<td>24.1 ± 1.9</td>
<td>13.2 ± 1.6</td>
<td>0.54 ± 0.01</td>
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<tr>
<td>30</td>
<td>25.2 ± 2.4</td>
<td>15.3 ± 2.5</td>
<td>0.60 ± 0.03</td>
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<tr>
<td>40</td>
<td>30.3 ± 3.6</td>
<td>17.1 ± 4.2</td>
<td>0.57 ± 0.03</td>
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<tr>
<td>50</td>
<td>32.1 ± 3.9</td>
<td>19.1 ± 3.8</td>
<td>0.59 ± 0.02</td>
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<tbody>
<tr>
<td>10</td>
<td>20.1 ± 2.5</td>
<td>12.3 ± 2</td>
<td>0.60 ± 0.01</td>
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<tr>
<td>20</td>
<td>22.1 ± 2.4</td>
<td>12.2 ± 3.5</td>
<td>0.55 ± 0.04</td>
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</tr>
<tr>
<td>30</td>
<td>24.2 ± 2.9</td>
<td>14.4 ± 2.5</td>
<td>0.58 ± 0.02</td>
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<tr>
<td>40</td>
<td>27.1 ± 3.8</td>
<td>15.1 ± 3.2</td>
<td>0.55 ± 0.03</td>
<td></td>
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<tr>
<td>50</td>
<td>30.2 ± 3.3</td>
<td>17.2 ± 3.8</td>
<td>0.57 ± 0.03</td>
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Liver specimens were sectioned in relation to the ablation area and the section of the liver where the ablation area was more obvious was photographed using a dimensional marker with a Leica Z6 APO (Leica Microsystems Srl; Buccinasco, Italy) stereoscopic microscope. Macroscopic examinations were performed to assess the absence of carbonization and tissue integrity. Morphometric analyses were performed on the image using the Image-Pro Insight v10.0.15 program to calculate the ablation area and the A and B dimensions—Figure 2a. After the initial morphometric analysis, a second cut was made halfway through the ablation with a scalpel to determine the centrality of the ablation—Figure 2b.

Macroscopic examination revealed whitish ellipsoidal areas due to ablation. In both sections, there was an indentation due to the probe. The blue lines correspond to the measurements taken using Image-Pro Insight v10.0.15 software. There were no brown-black areas due to carbonization.

The results obtained and presented in Table 4 are consistent with those documented in the literature and with the dimensions provided by other studies in metastasis ablation, as reported by Afaghi [36]. Certainly, the performance of the procedures can be significantly increased if the generator, with its thermal profile and power control algorithms, is combined with probes designed with advanced technologies and simulation systems. A good combination of this technology with state-of-the-art probes could significantly reduce procedure times, expose the patient to less risk, and maximize healthy tissue preservation.
2.2. Morpho-Histological Results

After macroscopic examination, a sample containing the ablated area and surrounding non-ablative tissue was taken from all areas of the ablated liver. A further ‘control’ sample was taken from an area of the liver away from all ablation procedures. Each section was embedded in paraffin and serially sectioned at 6–7 µm using a Leica RM2155 microtome. Sections were stained with H&E. Slides were observed using a Nikon Eclipse E600 (Nikon; Tokyo, Japan) biological microscope and photographed using a Leica DFC450 (Leica Microsystems Srl; Buccinasco, Italy) camera.

Morpho-histological analysis (see Section 3.5.2) shows that the MWA and RFA experiments confirmed the safety of the ablation procedures in bovine liver tissue. Macroscopic examination showed whitish ellipsoidal areas due to ablation; no carbonization was observed in the ablated soft tissue samples. Histologically, near the probe, there are some small areas of parenchymal destruction and some areas of coagulative necrosis due to the high temperatures achieved during the ablation process—Figure 3a. There was hyalinization and vacuolar degeneration of the cytoplasm around the disrupted area and the nuclei appeared slightly reduced and pyknotic—Figure 3b.

![Figure 3. Haematoxylin/eosin stained magnified images: (a) ablated liver 10×, (b) ablated liver 20×, (c) not ablated liver 20×. (a) shows areas of parenchymal destruction and areas of coagulative necrosis. (b) Hyalinization and vacuolar degeneration of the cytoplasm and slightly reduced and pyknotic nuclei compared to a normal liver shown (c).](image)

2.3. Discussion

Although the principle behind the effectiveness of the therapy is the same, there were significant differences between the MWA and RFA techniques used in this study. These differences need to be considered in order to make a rational choice based on the specific clinical case. The heating in the RFA method is due to the Joule effect associated with the current flow, which is based on the presence of dissolved ions in the fluids perfusing the tissue. This means that as the tissue dries, it conducts less and less current until it becomes charred as shown in Figure 4. For this reason, RFA should be supported by impedance control algorithms to avoid dehydrating tissue too quickly before the tumor reaches the desired temperature and necrosis.

Impedance monitoring in pre-clinical trials could help to calibrate the power delivery system and set an impedance alarm threshold that stops or minimizes the delivered RF power if the impedance trend is different from the expected one. In the MWA method, the MW transfers energy to the polar molecules of the tissue (mainly water), resulting in an increase in their temperature. This makes the technique less susceptible to drying phenomena and allows a larger volume to be heated uniformly in less time [37].

In the experiment shown here, the tissue was charred after a few minutes, making it impossible to complete the procedure. As the impedance increases, a higher voltage is required, which the RF generator cannot provide. To obtain this demonstration result, a small volume sample of chicken breast was used, so as to prevent the heat from dissipating and charring.
were associated with two different outputs and, depending on the applicator used for where it is necessary to achieve higher temperatures.

Very different shapes to better tailor the ablation pattern to the application and to reduce the very useful when it is desired to selectively ablate heterogeneous tissue, as the current will

carbonization was observed in the ablated soft tissue samples. Histologically, near the coagulative necrosis due to the high temperatures achieved during the ablation process—

Figure 3a. There was hyalinization and vacuolar degeneration of the cytoplasm around the disrupted area and the nuclei appeared slightly reduced and pyknotic—Figure 3b.

Each section was embedded in paraffin and serially sectioned at 6–7 µm using a Leica RM2155 microtome. Sections were stained with H&E. Slides were observed using a Nikon Eclipse E600 (Nikon; Tokyo, Japan) biological microscope and photographed using a Leica DFC450 (Leica Microsystems Srl; Buccinasco, Italy) camera.

Macroscopic examination showed whitish ellipsoidal areas due to ablation; no RM2155 microtome. Sections were stained with H&E. Slides were observed using a Nikon eclipse E600 (Nikon; Tokyo, Japan) biological microscope and photographed using a Leica DFC450 (Leica Microsystems Srl; Buccinasco, Italy) camera.

Figure 4. Impedance (blue) and temperature (red) trend in RFA.

As can be seen from Figure 4, the temperature of the tissue is not sufficient to determine the state of carbonization, so impedance monitoring is required to prevent the voltage output of the RF generator from reaching saturation.

While it may seem that MWA is always more effective, RFA conductive coupling is very useful when it is desired to selectively ablate heterogeneous tissue, as the current will always follow the path with the lowest impedance, e.g., effective for ablation of bone or spinal metastases [38]. Other advantages of the RFA are the ability to create applicators of very different shapes to better tailor the ablation pattern to the application and to reduce the size of the probe by eliminating the need for coaxial cables. Overall, RFA is less expensive and effective for performing controlled ablations with minimally invasive probes, such as cardiac pathologies, small lesions, and tissues with specific composition, while MWA is optimal for performing large ablations in highly vascularized tissues, such as liver cancer, where it is necessary to achieve higher temperatures.

3. Materials and Methods

The aim of this work was to determine the influence of different parameters related to RFA and MWA, which could help the operator select the most appropriate set of parameters for each clinical case. By observing the size of the ablated areas in the ex vivo tissues, i.e., chicken breast and bovine liver, in relation to the maximum MW or RF power, an ablation reference matrix was obtained. Correlation of the matrix with factors based on the tissue type and expected blood perfusion could be useful to guide the clinician in selecting the appropriate treatment. All the equipment mentioned in this section, including software, are custom products made by Leanfa s.r.l., Ruvo di Puglia, Italy.

3.1. Hybrid Microwave and Radiofrequency Generator

The hybrid generator used for the ex vivo testing, shown in Figure 5, is dual frequency and was designed to deliver MW power up to 250 W at 50 Ω at a frequency of 2450 MHz and up to 50 W of RF power at 100 Ω at a frequency of 480 kHz. The two frequencies were associated with two different outputs and, depending on the applicator used for the treatment, the two frequencies were used separately or in any combination. The unit could be connected to a pump for cooling the probes or the applicator, which could also be interlocked with the hybrid generator.

The hybrid dual frequency generator, cooling system, probes, and test procedures are described below.
3.1.1. Microwave Generator

The MW generator that is part of the hybrid dual frequency generator is shown in Figure 6. It operates at frequencies between 2400 MHz and 2500 MHz and provides up to 250 W of MW power. All the stages required for generation and amplification were installed in an aluminum cabinet mounted on an air-cooled heat sink. The selected frequency was generated by a phase-locked loop (PLL) oscillator capable of providing a stable signal over temperature variations. The signal was pre-amplified prior to the driver stage, which uses an LDMOS amplification system to raise the signal to a level suitable for the final power stage. All stages are temperature-compensated by a bias circuit. At the end of the amplification chain, there is the output circulator, which isolates the power stage from any reflected power due to mismatch conditions, protecting it and diverting any reflected power to an internal load. A directional coupler made it possible to measure the forward and reflected power; the data could be used in different ways, by different algorithms, to achieve a level of control precise enough to maintain a stable power output over time and with increasing temperature. The algorithms could be used to protect the integrity of the MW generator and ancillary systems, such as the catheter. These control systems can be digital or analog, in order to be able to react quickly to certain hazardous scenarios. The fact that a solid-state technology allows the power to be delivered at a single frequency, which must be matched to the connected load, allows maximum energy transfer from the generator to the load.

Figure 5. Photo of the hybrid dual frequency MW + RF generator in an experimental setup for MWA performed on chicken breast.

Figure 6. Schematic of the solid-state MW generator and control loop.
3.1.2. Radiofrequency Generator

The RF generator shown in Figure 7 operates at a frequency of 480 kHz and can deliver up to 50 W of RF power. The frequency is generated by a quartz oscillator and amplified by two different gain chains to provide the power signal for therapy and the pilot tone for impedance measurement. The potential-free output is provided by the isolation transformer to reduce the risk of creating alternative current paths, which could lead to unwanted burns in the load.

![Figure 7. Schematics of the RF generator.](image)

3.2. Probes

Thermal ablation aims to heat the target tumor to a temperature of >60 °C to denature its proteins and induce coagulative necrosis of the cells. The technological challenge is to heat up the entire volume occupied by the tumor in the shortest possible time, while trying to preserve the surrounding healthy tissue. For this reason, the most typical application uses needle or catheter probes introduced into the target tumor by minimally invasive surgery [39]. The design of the probe varies according to the frequency and technologies used.

3.2.1. Probes Working Principles

The RF probe used in this study consisted of two cables that passed through the applicator to connect to two electrodes whose size and spacing determined the shape of the ablation volume. The MW probe consisted of a coaxial cable and a special tip that acted as an antenna.

It should be noted that due to the unbalanced nature of coaxial cables, current can flow along the surface of the outer conductor, causing unwanted heating along the cable [40]. Therefore, the antenna must be properly matched to the load for maximum energy transfer and must have a system to suppress current flow along the outer conductor. In the literature [41–44], there are various methods that can be used to suppress RF currents due to reflection, but for simplicity, in order to be able to read the MW reflected power, the length of the coaxial cable inside the probe was defined empirically based on minimizing the read reflected power value. The probes were monitored by two thermocouples—Figure 8c—one located near the tip of the probe to monitor the ablation temperature and one located immediately after the ablation zone, where the temperature must not rise for any reason, to protect tissue outside the target treated volume.
3.2.2. Probes Cooling

To avoid heating along the probe body (the path from the handpiece to the tip), which could cause serious tissue damage, a cooling system was embedded in both probes (RF and MW). The cooling system consisted of two thin concentric tubes as shown in Figure 8b. The inner tube carried the cooling fluid (water) from the handpiece to the probe tip, while the return water flowing through the outer tube cooled the probe shaft. The flow of cold fluid (blue arrows) passes through the inner pipe, interacts with the hot zone by exchanging heat (orange arrows) and returns back to a higher temperature (red arrows) through the outer pipe. A peristaltic pump was used to flow the required water through the cooling circuit at constant pressure and flow—Figure 8a.

3.3. Target Tissues

In order to test ablation and collect data on the difference between the two treatments, it was necessary to use a tissue that closely resembles real cases of treatments in humans. Chicken breast and bovine liver, as ex vivo tissues, offer distinct advantages in RFA and MWA testing. Chicken breast is useful for assessing heat distribution and energy penetration in uniform muscle tissue, while bovine liver provides a more realistic model for the ablation of vascularized tissue, similar to human liver. The use of both tissue types allowed a comprehensive and effective evaluation of both ablation technologies in terms of safety and efficacy.

3.4. Ablation Tests

The ablation tests consisted of setting a maximum power that the generator could not exceed during the procedure. An ablation temperature was also set. A control algorithm using the temperature feedback read by the thermocouple mounted on the tip of the probe, as shown in Figure 9, allowed the temperature of the target tissue to be brought up to the set value with a ramp of 1 °C/s and then kept constant throughout the procedure.
The controller used was a modified version of the PID, where an error between the setpoint and the actual temperature value was calculated. This was used to derive a target power level, which was then processed by each individual generator to provide a stable power value regardless of the load. Temperature errors were also limited and weighted with different constants depending on whether the energy used was MW or RF, and whether the algorithm was doing the heating or keeping the temperature at the set target.

Procedures were performed using a closed-loop temperature control algorithm with the maximum output power set and the procedure duration fixed at 10 min. The algorithm ran a constant temperature ramp until the target was reached. As protein denaturation begins at around 60 °C and the boiling temperature of water is around 100 °C, a good target to set was 95 °C to heat the tissue as much as possible without creating bubbles that could mismatch the load in MWA or reduce the contact area with the RF electrodes in RFA. In the following tests, the maximum power used was changed from minimum to maximum available power in 5 steps. The difference between the different trials was therefore related to the management of the peak power modulation to achieve the same temperature profile.

3.5. Ablation Evaluation Methods

Validation of ablation procedures in the laboratory may involve various tests and evaluations to ensure the efficacy, safety, and consistency of the treatment. Among the various validation techniques, the present study used dimensional analysis of the shape of the ablated area and the histological analysis of the treated tissue.

3.5.1. Roundness Index

The parameter used to evaluate the shape of the ablated area, in addition to the diameters and the ablation volume, is the roundness index (R.I.), described by Park et al. [45], and then used as a standard metric to evaluate the goodness of the ablated shape [46–48]. Park defines the R.I. as the ratio between the two axes of the ellipsoids, as shown in Figure 10.

Figure 9. Schematics of the closed-loop temperature control algorithm.

Figure 10. Ablation diameters used to calculate roundness index.
Assuming that the treated tissue is homogeneous and given the difficulty of measuring both the transverse and longitudinal diameters due to the incision that must be made on the ablated tissue, it is reasonable to assume that the two are equal, as shown in Figure 10. This leads to the planar R.I. being considered the most representative of the sphericity of the ablation. On the other hand, if the other axes of the ellipsoid were taken into account, we would obtain R.I. values very close to 1, making it difficult to compare the different results.

The R.I., defined in Equation (1), has a value between 0 and 1, since the axis of the needle is by nature always greater than the other two; a high R.I. value represents a tendency towards a spherical shape (if R.I. = 1, the two axes are identical) and can be calculated as the ratio between two ablation diameters.

\[
\text{R.I.} = \frac{\text{Size B}}{\text{Size A}}
\]

### 3.5.2. Histological Imaging

By obtaining and analyzing tissue samples from the ablated area, histological analysis provides detailed information at the cellular level. The method uses stains such as hematoxylin and eosin (H&E) to highlight cellular structures and differences between normal and necrotic tissue, identify signs of coagulative necrosis, and assess the boundary between ablated and non-ablated tissue, providing an accurate assessment of ablation effectiveness. Necrotic cells often show a change in morphology compared to viable cells. This can include loss of cell contours, cell shrinkage (pyroptosis), and destruction of the plasma membrane.

### 4. Conclusions

For medical applications of both microwave and radiofrequency radiation, i.e., radiation whose energy is converted directly into heat within tumors, the parameters to be considered are related to the tumor, e.g., volume/mass to be treated, and to the equipment, e.g., frequency, type of generator, energy transfer system, and its control. One of the key points in the development of adapted ablation equipment and its use is the introduction of solid-state generators and the precision of their frequency and power delivery capabilities, together with higher process control capabilities.

The hybrid dual-frequency microwave and radiofrequency generator used in this research has demonstrated increased accuracy due to its proprietary hardware, which incorporates technologies and algorithms to control the emitted electromagnetic power vs. temperature profile of the treated sample. In particular, the hybrid generator incorporates closed-loop algorithms. In addition, control systems based on impedance or reflected power measurements monitor the ablation to avoid unwanted carbonization and to include areas where tissue damage is not desired.

Now that the efficacy of radiofrequency and microwave ablation has been proven and the number of procedures has increased over the years, it is important for the medical industry to focus on improving the performance of these procedures. For radiofrequency ablation, it is necessary to invest in delivery algorithms according to electrical parameters related to tissue hydration. In addition, multi-probe applications can be a good way to increase the volume to be treated with the same procedure time. For microwave ablation, it is necessary to invest in antennas that allow greater customization of ablation shapes and control of the high temperatures that can be achieved with this technology. The issue of higher costs may be offset over time by a reduction in hospital stay and the likelihood of metastatic recurrence. These improvements can be combined to achieve the best results for each technology, due to the use of hybrid generators that allow specific therapy for each case. Following ablation by both radiofrequency and microwave techniques, it would be important for the medical industry to focus on improving the performance of the probes to detect more histologically evident necrosis.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The data presented in this study are available on request from the corresponding author due to Leanfa s.r.l. intellectual property.

Conflicts of Interest: Fabio Lobascio, Rocco Di Modugno, Marco Fiore, Nicola Di Modugno, Cristian Bruno and Thomas De Nicolò are Leanfa employees; Rossella Veronica Barberis and Karine Cabiale are employees of Life and Devices, while Marilena Radoiu is an employee of Microwave Technologies Consulting. The authors who carried out this study declare that they have no mutual conflicts of interest and that the funders of the companies mentioned had no role in the design of the study; in the collection, analysis or interpretation of data; in drafting the manuscript; or in the decision to publish the results.

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