# BALLOON MODEL FOR WATER VAPOR ABLATION OF NON-MUSCULAR INVASIVE BLADDER CANCER CELLS

By

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# ABSTRACT

Bladder cancer has a considerable issue with multiple recurrences of tumors, and current treatments are unable to adequately address these concerns. This results in continuous surveillance. Ablation, or killing, of bladder cancer cells is often incomplete and results in a high probability of recurrence. This paper aims to evaluate a potential method of ablation using a model that simulates a bladder and what conditions are needed for optimal quality and depth penetration. This balloon model for water vapor ablation is intended to simulate bladder cancer cells and determine if this is a viable avenue for future research.

A device from Francis Medical, in combination with mylar balloons and raw chicken, was used as the equipment to support a model to determine the viability of water vapor ablation for bladder cancer. This was achieved using raw chicken in mylar balloons to simulate the surfaces in a bladder. Water vapor is injected into the balloon, and the outer surface of the chicken undergoes energy transfer. After treatment, the chicken was evaluated to see how far the energy had penetrated the tissue and what ablation quality had occurred.

After conducting several experiments at various durations and energy levels, it was found that the chicken achieved its ideal depth when it was treated for 6 seconds at 50 calories per second. Based on the multitude of experiments, the ultimate determination was that while it will require more stringent modeling and experimentation in the future to explore the possibilities and viability, this method merits future study. It efficiently and effectively transferred energy to kill a uniform layer of cells on the surface of the chicken.

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# INTRODUCTION

Bladder cancer is the sixth most common cancer in the United States and the tenth most common cancer in the world [1, 2]. Just under 200,000 people died from bladder cancer in 2018 [3]. Despite its prevalence, current treatment options still leave much to be desired. Utilizing water vapor to ablate or kill bladder cancer cells within the bladder could be a potential future treatment option that is less invasive and requires fewer treatments. This thesis investigates the viability and dosimetry of a water vapor generator and ablation catheter delivery device in a laboratory setting.

## Bladder Cancer

### Facts and Stats

Bladder cancer, like all cancers, can be a devastating disease, and treatments can be extremely challenging for the patient. Treatments are complicated since bladder cancer overwhelmingly affects the elderly population. Treatments aim to be as painless, effective, and non-invasive as possible. While treatments are constantly improving in these areas, the long-term effects of chemotherapy chemicals used in the treatment process are a considerable concern. An ideal treatment would address all of these issues.

Another concern is that bladder cancer is commonly reoccurring. The National Library of Medicine notes that, “Bladder cancer tends to recur, even when it is non-invasive at the time of diagnosis; therefore, standard practice is to perform surveillance of the urinary tract after a diagnosis of bladder cancer” [1]. After a diagnosis of bladder cancer, even one that is minimally invasive, constant surveillance via cystoscopy will be necessary for the remainder of the patient’s life. Even more aggravating, there is no widely accepted screening method for bladder cancer recurrences [3]. Patients must undergo cystoscopy regularly to ensure their disease has not had a

resurgence. Over 70% of patients will experience a recurrence within five years of their initial diagnosis, with 45% seeing progression [3].

Bladder cancer primarily affects the population above 55 years of age, with the average age of diagnosis being 73 years old. As the global average age increases due to improved healthcare, bladder cancer diagnoses will continue to rise [1]. Because the average age is much higher than other diseases and with its propensity towards recurrences, bladder cancer has another unique danger. Procedures under general anesthesia grow more dangerous with the age of the patient. The geriatric population is at an especially high risk of morbidity and mortality, especially when the procedure must be repeated [4]. Treatments requiring little to no general anesthesia are preferable to ensure the safety of the patients.

Other than age, there are several other risk factors. The most common risk factor in the United States is tobacco use [1]. It is more likely to affect men than women [2]. Exposure to carcinogenic chemicals, particularly for electrical and chemical process workers, poses a high risk [3].

**Structure of the Bladder**

The bladder is a natural chamber in the body used to process, hold, and then eliminate urine. When empty, it's roughly tetrahedral-shaped [5]. The typical bladder volume when the bladder is full is around 500 milliliters [6]. The bladder is approximately the size and shape of a pear when filled and is connected to the upper parts of the urinary tract via ureters from the kidneys [7]. Urine travels from the bladder and out of the body via the urethra. The overall structure of the bladder is shown in Figure 1 [8].

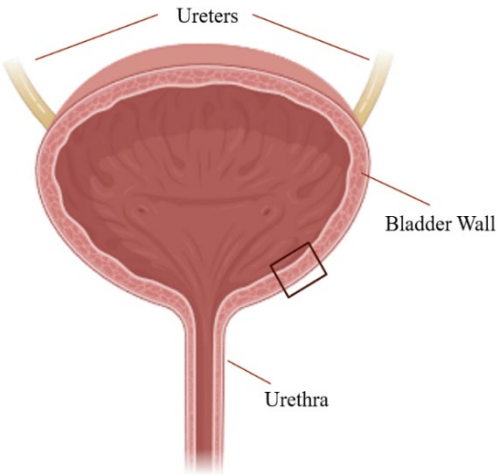


*Figure 1 Diagram of the bladder [8]*

A vital key about the bladder is that much of its size and shape are determined by factors such as volume, level of expansion, and the location and distance of nearby organs [5]. Its most unique feature is its ability to use "coordinated contraction and relaxation to facilitate its physiological function" [6]. The position within the body is reliant on the volume, with a full bladder extending into the abdomen but an empty one remaining solely within the pelvic region [5].

The other structural element critical to the discussion of bladder cancer is the structure of the bladder's interior walls. The wall thickness is capable of significant change to adapt to various levels of expansion. The bladder interior wall consists of three layers: mucosa, submucosa, and muscularis [7].

The innermost layer is the mucosa, also known as the transitional epithelium or lining epithelium [6]. This layer contains no blood or lymphatic vessels and consists of several layers of cells that reorganize when the bladder expands. Its primary function is as a protective layer and barrier.

The middle layer is the submucosa or lamina propria. This thin layer contains blood and lymphatic vessels and is the first layer from which cancers can metastasize to other parts of the body. The outer edges of this layer interlace with the muscular layer. It acts primarily as a connective layer between the mucosa and the muscularis.

The muscularis, or muscular layer, is the outermost layer. It is subdivided into three additional layers: inner longitudinal, middle circular, and outer longitudinal [7]. This layer is the thickest and is the driver of contraction and expansion [9]. A close-up illustration of these layers is shown below in Figure 2 [10].

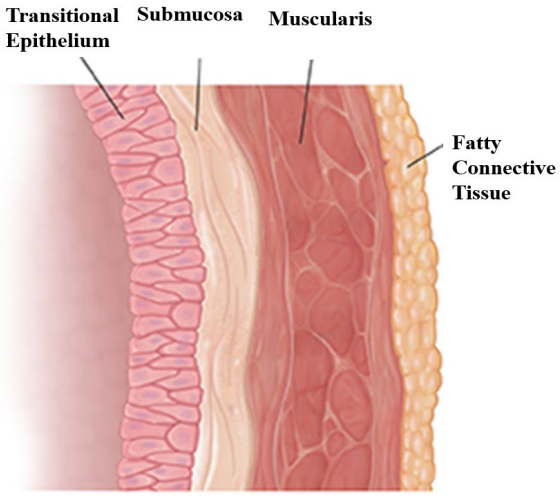


*Figure 2 Bladder wall diagram [12]*

As discussed above, the bladder has an incredible ability to stretch and contract. Much of that ability relies on the structure of the bladder wall and its ability to become thinner as needed. The thickness of the wall can differ based on the volume inside the bladder, from as thin as a mean of 2 millimeters in a relatively full bladder to a mean thickness of 4.6 millimeters in an empty bladder [9]. Males have a slightly thicker bladder wall, but the difference has not been found to have clinical significance [11].

The mucosa layer is a unique structure with distinct cell morphologies and properties. The innermost part of the lining consists of umbrella-like cells, called urothelial cells, that prevent the penetration of urine into the inner layers [12]. These cells are one of the most distinctive features of the bladder and have proven difficult to replicate in laboratory studies.

The urothelial cells in the innermost layer have an incredible ability to respond to injury to the bladder. While under normal, healthy conditions, the bladder wall has a slow turnover for new cells. However, when under stress, particularly due to injury, the adult urothelium has the ability to have a "complete restoration of newly differentiated superficial cells within 72 hours [13]."

The specific properties of the bladder are challenging to determine, and they must be quantified at a multitude of expansion levels. In recent studies, it has been determined that the bladder wall thickness has a hyperbolic relationship with volume until reaching a plateau at around 200 milliliters [11]. However, the alignment and orientation of cells changes dramatically throughout the initial stages of expansion.

**Types of Bladder Cancer**

Bladder cancer can be broadly separated into two categories: muscular-invasive (MI) and non-muscular invasive bladder cancer (NMI). Either of these categories may manifest as a high-grade (aggressive) or low-grade cancer [1, 3]. However, 95% of invasive carcinomas are high-grade [14]. More than 90% of bladder cancers are transitional cell carcinomas derived from the uroepithelium. The presentation and grade of the tumors can be quite varied [1].

Muscular-invasive bladder cancer occurs when the tumor has infiltrated the muscular layer. This variety is the most dangerous type of bladder cancer. The majority of muscular-invasive bladder cancer patients will die from bladder cancer within two years of diagnosis [3].

The muscular layer contains both blood vessels and lymph vessels, which facilitates the metastasis of the tumor to other parts of the body [7].

Non-muscular invasive bladder cancer is the most common variety of bladder cancer. While less deadly than muscular-invasive, it can still be fatal, and it is highly likely to reoccur. Over 70% of patients experience a reoccurrence within five years of the initial diagnosis, and 45% see progression [3]. This variety of bladder cancer is the primary focus of water vapor ablation and of this investigation. It is on the interior surface of the bladder and can be ablated without damaging any submucosa nerve or structural proteins. Since non-muscular invasive bladder cancer is the most common form, improving patient outcomes will enhance outcomes for the majority of patients overall.

According to the World Health Organization's official classification, non-invasive tumors can be further divided into papillary or flat. Cancers without any papillary structures are called carcinoma *in situ* (CIS) and are typically high-grade cancers [14].” Despite being high-grade, carcinoma *in situ* would be a viable candidate for investigation for treatment with water vapor ablation. It tends to spread across a flat plane, making it difficult to address through current treatments due to the inconclusive boundaries, which makes excision difficult.

**Stages of Bladder Cancer and Classification**

Features of a tumor that are typically taken into account for prognosis for non-musclular invasive carcinomas include the depth of invasion into the bladder wall, pathological grade of the tumor, presence versus absence of carcinoma *in situ*, number of tumors, tumor size, invasion of the lamina propria, and whether this was a primary tumor or recurrence [1]. In clinical settings, bladder cancers are typically classified by TNM (tumor, node, metastasis) [1]. These classifications are then utilized to give the cancer a stage, which is used to discuss treatment

options. The stages range from 0 to IV, with more specific stage classifications based on the variety of cancers present. It is worth noting that there may be a variety of histological types even within a specific stage of bladder cancer [15].

The focus for water vapor ablation would be primarily on Stage 0 bladder cancers, with Stage I cancers as a possibility pending further research. Stage 0 comprises of noninvasive papillary carcinoma and urothelial carcinoma *in situ* in cases where there is no evidence of lymph node metastasis or distant metastasis. Stage I will require some additional research into the lamina propria, but as it only includes tumors that have invaded the lamina propria and have not yet invaded the muscularis and have no evidence of either lymph node or distant metastasis, it may still be a potential stage that could be treated with water vapor ablation [1].

**Current Treatments**

Current treatments for bladder cancer depend on the type and severity of the case. Higher-grade cancers will require more aggressive treatments. The most aggressive and high-grade cancers require full or partial removal of the bladder, a procedure called a radical cystectomy. This treatment is followed by one of several varieties of chemotherapy. These varieties of cancer are beyond the scope of this device.

The typical treatment for non-muscular invasive bladder cancer at Stage 0 or I is transurethral resection of the bladder tumor or TURBT [1]. This procedure involves a surgeon inserting a surgical loop through the urethra that can cut tissue sections. For smaller tumors, they can remove the entire lesion at once, but for larger tumors, it requires cutting the tumor into samples that are removed separately. For especially large tumors, surgeons will only take a sample for biopsy [16]. After biopsy, an en bloc resection of bladder tumor (EBRT), or a segmental or radical cystectomy may be required [1, 17].

Because TURBT has a considerable risk of recurrence, most patients will then undergo intravesical chemotherapy. There is no current consensus on the cause of the constant recurrences from TURBT treatments. The most widely accepted explanation is that TURBT does not adequately remove all of the cancer or that during its removal, parts of the tumor can reattach in new locations [18].

Much of the current literature is focused on analyzing different after-care methods, such as determining the best combination of chemicals for intravesical chemotherapy [19]. Another significant area of research is focused on molecular markers and the development of more personalized treatments [20]. Because there is such a difference in the responses of seemingly similar bladder cancer, the histology and mechanisms of many of the common forms of bladder cancer are being documented to determine the best treatment for each patient.

This current research focus presents an opportunity for this investigation since it represents a complementary exploration to current research rather than a radically novel approach. If the initial removal of the tumor is more effective at eradicating all traces of the bladder cancer cells, it improves the patient's outcomes. Supplementary treatments, such as intravesical chemotherapy, could still be used to reduce recurrence risks further.

There are some current efforts to utilize other methods of ablation, such as laser ablation [21, 22]. However, many of these methods rely on conductive heat transfer, meaning they cannot address the entirety of the lining all at once and cannot ablate as uniformly. These methods do have some of the same advantages as water vapor ablation, specifically being outpatient procedures and not requiring general anesthesia [21].

Early detection would be extremely helpful in lowering the overall risk, but even now, there are not any diagnosis tools that can detect cancer before the onset of symptoms [23].

Cystoscopy is an effective diagnostic tool but is typically only used when symptoms arise. However, symptoms often arise well after bladder cancer has become established. The reliance on regularly undergoing cystoscopy procedures presents another reason why screening for recurrences can be so difficult. However, improvements in genetic testing and its availability could be utilized to identify individuals with genetic predisposition [1].

## Water Vapor Ablation

Water vapor ablation, in the simplest terms, means using the release of energy from the condensation of water vapor to kill cells. The amount of energy present in a single gram of water increases substantially when it moves from the liquid to the vapor state. The amount of energy held in liquid water is equal to 1 calorie per gram per °C while the energy held in water vapor is equal to 539 calories per gram at 100°C [24]. The change in thermal energy of water vapor can be seen in Figure 3 [24]. This difference in energy allows a large amount of energy to be released when water condenses. When water vapor condenses on a tissue, energy is released, disrupting the cell membranes and causing cell death or ablation.

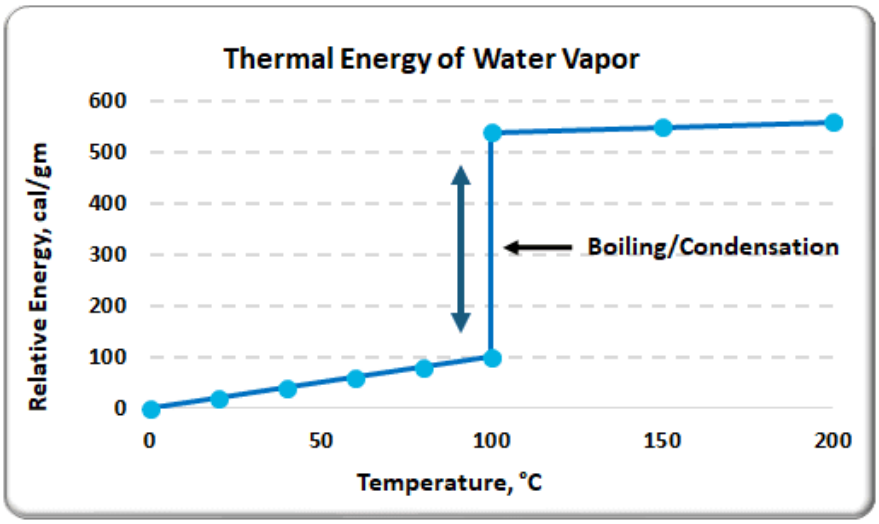


*Figure 3 Thermal Energy of Water Vapor [22]*

Water vapor ablation has already been utilized in other applications within the body. Notably, the Rezūm® system acts as a treatment for benign prostate hyperplasia (enlarged prostate), and the Mara™ Water Vapor Ablation System aids in the treatment of menorrhagia or heavy menstrual bleeding [25, 26]. Both treatments have completed the Food and Drug Administration's clinical trial requirements and are available for use in patients. These treatments claim short but effective treatments. Mara™ treatments are estimated to take around 2 minutes, while Rezūm® has a nine-second treatment time. Determining the source of this disparity in treatment time for ablation would be necessary to determine realistic expectations for ablation within the bladder. Significant observational and clinical studies have shown both treatments to be safe and at least as effective as alternative methods of treatment [27, 28].

Ablation technology is not currently used clinically for any cancers. Cancerous cells' morphology and reactions may differ from normal cells, which will be a future research target. In addition, while the other organs are also made of epithelial cells, the epithelial cells in the bladder are specifically transitional epithelial cells, which are unique to the urinary system. Their role as a liquid impervious barrier may present additional difficulties in clinical treatment and would require additional research.

**Mechanism**

The energy source in this process can be considered heat in the colloquial sense, but in truth, the source is entropy. Entropy is a measure of the disorder within a system; in a medium such as a gas, there are greater degrees of freedom, and the entropy increases. Under standard ambient conditions, the entropy of water is 16.8 calories per kelvin per mole [29]. As an ideal gas at 298 K and atmospheric pressure, the entropy of water vapor is 31 calories per kelvin per mole [29]. This difference is due to increased translational degrees of freedom, which contribute

twice as much in the gas state. When the gas condenses, this drop in entropy requires an outlet for that excess energy.

Another important mechanism of this method of water ablation is the utilization of convective heat transfer, which allows for the penetration into interstitial spaces [24]. When discussing living cells, the penetration will be more uniform as the dispersion of water vapor and heat transfer will occur across and between all surfaces simultaneously rather than radiating from a central touch point.

## Objective and Specific Aims

Current treatments for bladder cancer cannot address recurrence and are harsh on patients' bodies. Water vapor ablation could be a reasonable treatment possibility, but current research has yet to fully explore its utility. Researching the potential for water ablation of the bladder could unlock a new, effective, and far more humane treatment for bladder cancer.

The ultimate intention for this technology and the basis for the model in this thesis is shown in Figure 4. Using a catheter, water vapor would be injected into the bladder and circulated. When it condenses on the interior surfaces of the bladder, it releases energy, killing a uniform layer of cells on the surface of the bladder, including any cancer cells. 70% of all bladder cancer is on the interior surface; therefore, only a depth of 1 millimeter is required to eradicate virtually all bladder cancer cells of the target types and stages. By using water vapor circulation, all the cancer on the interior surface of the bladder will be ablated at once rather than treating it one spot at a time, potentially missing some cancer cells.

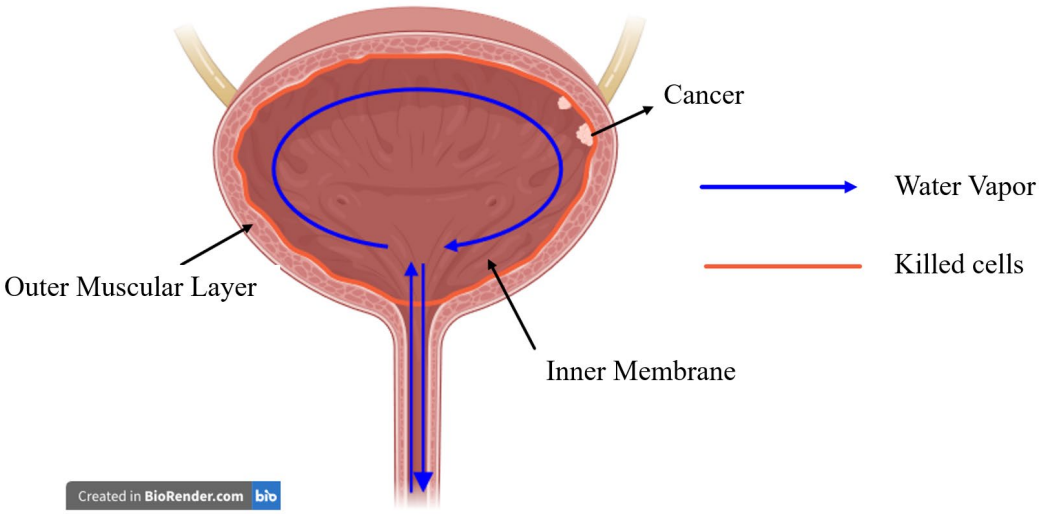


*Figure 4 Basis for Experimental Model*

**Objective**

This project aims to determine the viability and dosimetry of a water vapor ablation treatment for bladder cancer cells in a laboratory study.

**Specific Aims**

1. Determine the relationship between treatment time and depth penetration and definition.
2. Determine the relationship between treatment energy and depth penetration and definition.
3. Determine the treatment time and energy required for a consistent and uniform 1-millimeter depth penetration in chicken.
4. Determine the viability of this setup for continued research and note potential issues for expansion and future models.

**RESEARCH METHODOLOGY**

An analog experimental model was developed to evaluate the Francis Medical water vapor ablation device's capabilities. The overall idea of this model was that a mylar balloon

could be used as a stand-in for the bladder, and a sample of chicken could be placed inside to determine how far into the bladder wall the treatment would penetrate.

This water vapor delivery method's most essential and unique feature is the water vapor's ability to circulate the tissue convectively. This ability allows better and more uniform energy transfer, so the model needed to accommodate this circulation.

## Experimental Setup

Figure 5 is a simplified schematic of the model used. Distilled water went to a syringe controlled by a computer system that had several adjustable settings. The computer system also controlled the steam generator. Distilled water was flushed through the system to set up the water vapor generator. The setup could not be run dry, as that would damage the devices and sensors. After coming out of the steam generator, the inlet tube with the water vapor went into a mylar balloon, which was cut into the approximate size and shape of the bladder.

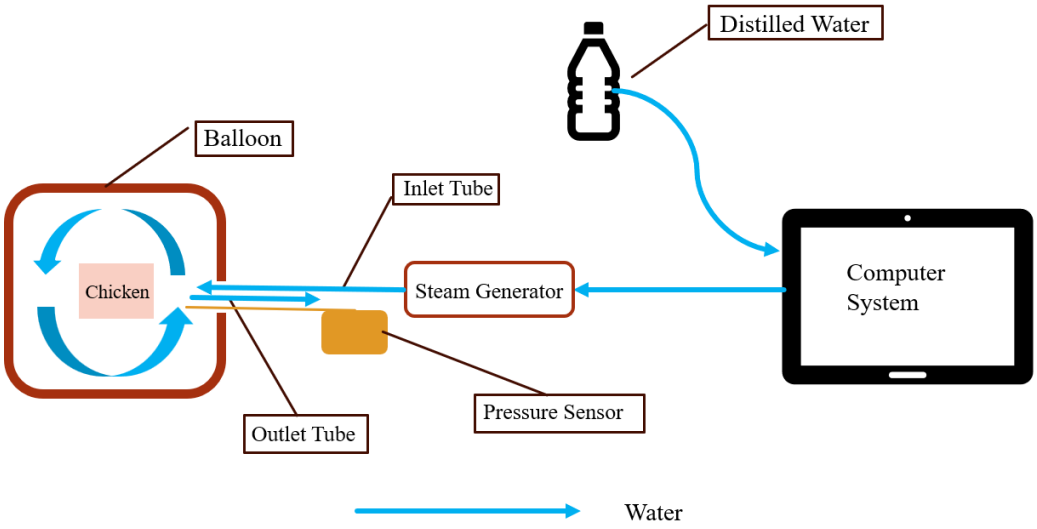


*Figure 5 Simplified schematic of the setup.*

After circulating, any excess water vapor exited through an outlet tube. This prevented overinflation. Sealed inside the balloon was a sample of raw chicken. Just like in the bladder, the

water vapor would circulate, and as it condensed on the surface of the chicken, the thermal energy was released, and the depth of the thermal energy penetration could be determined by the difference in the color of the chicken before and after the treatment. A pressure sensor with live readings was used to prevent high pressures that a real bladder would be unable to withstand.

Figure 6 shows the practical experimental setup without a balloon attached. This was the actual setup used for this experiment.

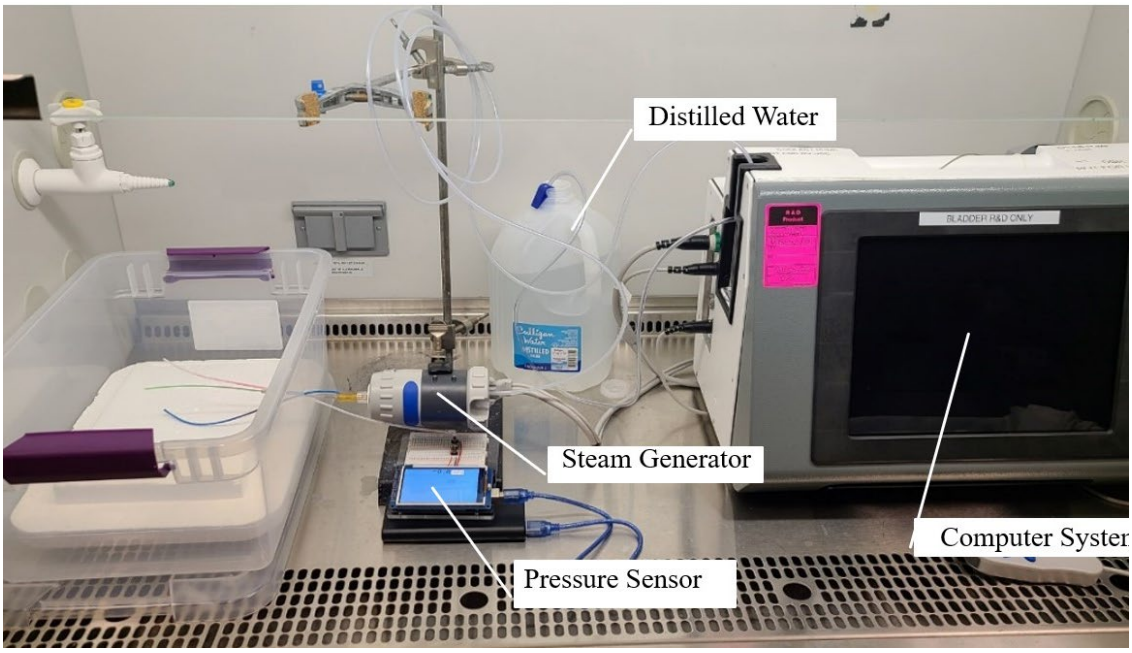


*Figure 6 Complete experimental setup*

The screen is a simple touchscreen with a settings panel that can easily change the different parameters.

The steam generator produced water vapor at a specific flow rate through the inlet tube that, along with an outlet tube and a pressure sensor tube, was threaded through a hole in a plastic bin and into a balloon. For convenience, the steam generator was placed on a ring stand to maintain a consistent height. A labeled view of this part of the setup can be seen in Figure 7. In Figure 7B, the same setup is shown, but with a balloon attached. The balloon has a small sample

of chicken sealed inside. For earlier experiments, a voltmeter was set up with a pressure sensor to show the current pressure inside the balloon via a conversion from the volts. The conversion table from volts to pressure employed is available in the appendix. Later experiments utilized a pressure sensor setup that displayed the pressure in mmHG, which is the iteration seen in Figure 7.

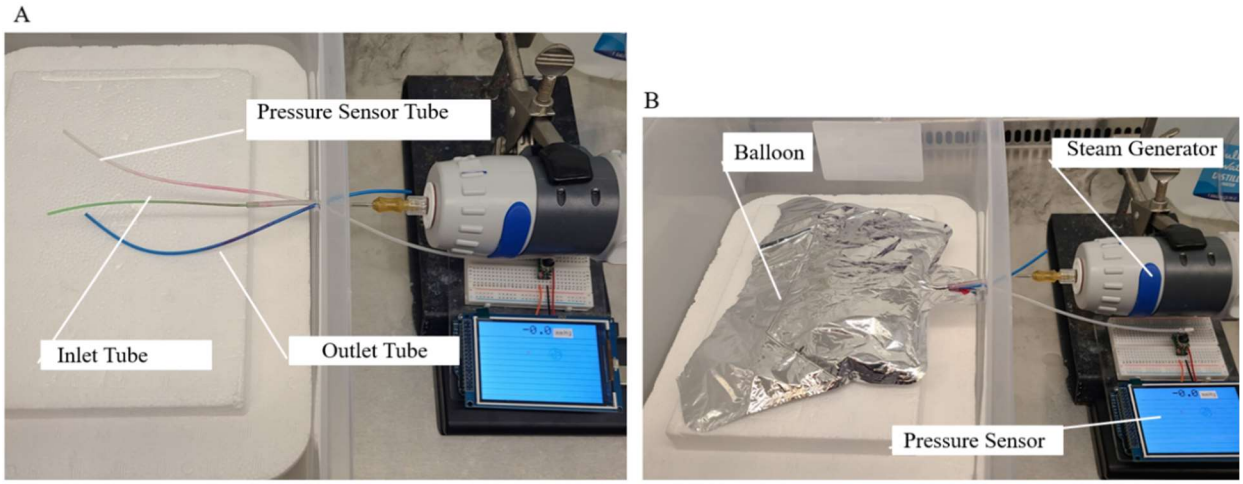


*Figure 7 A. Steam generator and bin setup without a balloon. B. Steam generator and bin setup with a balloon*

**Computer System and Settings Setup**

Once the system had been set up, the programmable features were set to the parameter of the current experiment. The computer system used had several settings that could be adjusted, which were the investigation's primary focus. It could set two distinct phases or stages: an inflation phase and a treatment phase. Each of these phases had adjustable settings for the duration and for the energy. Note that the energies are a flow rate and are given in calories per second while the time is in seconds.

The goal for these two phases was that the initial inflation stage would have high energy and low time to quickly inflate the balloon to the desired pressure. Then the treatment period would begin with a lower energy sustained for a longer time, so it could then maintain a steady pressure that was initially reached during the inflation stage. Changing these settings was the

primary way that experiments were changed and the ultimate goal of these experiments was to determine what settings gave the optimal results.

Table 1 shows the default values for the programmable factors in the computer system. Francis Medical set default settings for this experimental setup based on their previous studies. For many early experiments, the values for the inflation settings (inflation time and inflation energy) were not changed, as the values for treatment settings were expected to be the most impactful. This assumption was later challenged, and the values, especially for inflation time, were changed in several of the later experiments. The offset setting was meant to change the quality of the steam, making it "drier" or "wetter." However, based on information from Francis Medical, the offset settings were not adjusted as they had been set to the parameters they had found most successful in their previous investigations. The offset was only to be adjusted if there were issues. Nonetheless, this could be a potential source for future investigations.

*Table 1 Default values for the computer system*

| | **Time** | **Energy** | **Offset** |
|---|---|---|---|
| **Inflation** | 10 secs | 50 cal/sec | 0 |
| **Treatment** | 20 secs | 30 cal/sec | 0 |

**Microscope and Analysis**

After treatment, the chicken was promptly removed from the balloon to prevent additional energy transfer and placed on a cutting board. A section from the center of the chicken was removed, and a macro photo of both sides and the center portion was taken with a phone camera. A representative image of this type of macro picture can be seen in Figure 8.

*Figure 8 Representative cross-section of treated chicken*

The macro photo was used to determine a qualitative score from one to ten on how well-defined the border between the parts of the chicken that were ablated and the areas that did not experience any energy transfer. An example of this scoring is shown in Figure 9.

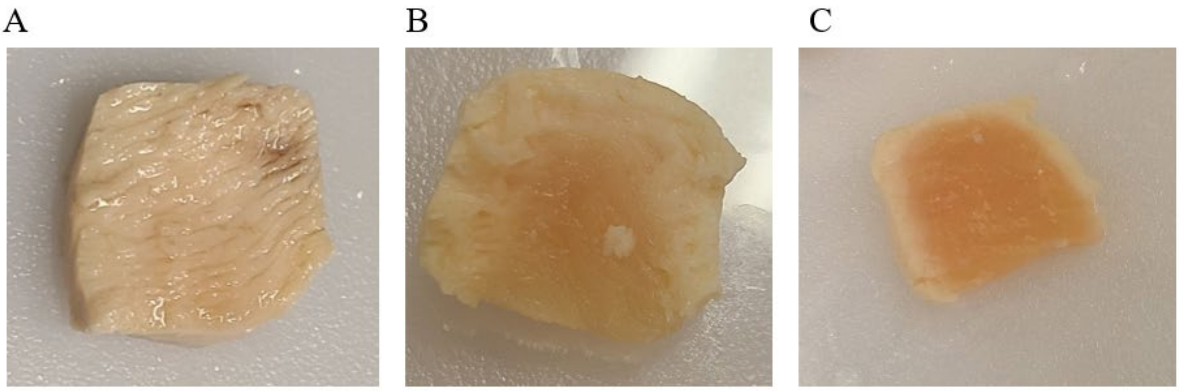


*Figure 9 Example scoring metrics for macro photos. A = 1, B = 5, C = 10.*

A would score a one because there is no discernible difference between the treated and untreated sections. B would be a five because while there is a noticeable difference between the treated and untreated portions, there is a lack of definition, which makes it difficult to pinpoint exactly where the treated area ends. C would be a ten because it has a clearly defined line between the treated and untreated chicken and is uniform on all three exposed sides.

Definition as a metric was important because analyzing the mean depth penetration alone did not adequately address the entirety of variations within the penetration. While two chicken samples might have the same average depth penetration, a more uniform and defined penetration was preferable. There are several layers in the bladder wall, so treatments must not penetrate too far and risk damaging layers that would not regenerate the same way as the innermost layer would. Ideally, treatments should be uniform and controllable.

The center portion was then captured under an Olympus SZX10 microscope equipped with a DP22 digital camera using cellSens. Supplementary lights and white balancing settings ensured reasonable contrast. The microscope's magnification was set to 0.63. All images were analyzed using default settings in ImageJ's software. The scale was determined image by image to ensure that the measurements were accurate. Figure 10 shows a representative example of the labeled ImageJ measurements.

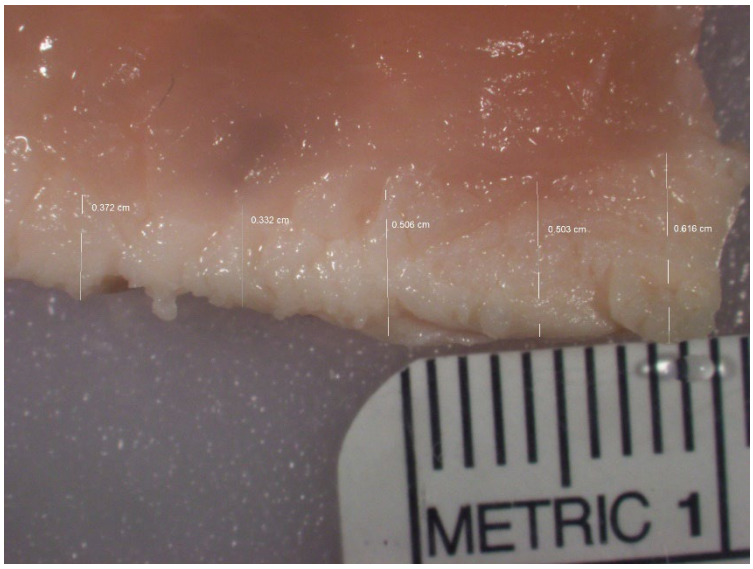


*Figure 10 Sample of chicken under a microscope with labeled distances*

Five measurements were taken on each of the three treated sides of each chicken sample. Note that one side would be interfacing with the balloon and would have no circulation around it, thus having no energy transfer.

These measurements were then added to an Excel spreadsheet, and each side's mean depth penetration and the run's overall mean were determined.

## Pilot Studies

Several experiments were performed within this larger experiment, which are explained in detail further in this thesis. The rationale for many decisions was determined using pilot studies, which will be discussed in this section. A critical goal of this investigation was to determine and refine the parameters for future experiments.

### Outlet Size

The first completed pilot test was injecting water vapor into the balloons without any chicken to verify that all parts of the setup were working and to see the general threshold for the balloon to pop. This experiment utilized several different outlet sizes and energies and was a straightforward determination of whether the balloon popped. The outlet sizes used were a 14-gauge outlet and a 7-French gauge outlet. These outlet sizes were chosen for their convenience and availability. The default inflation energies noted in Table 1 were used, and a 20-second treatment time. The treatment energy was increased incrementally until the balloon popped or the top setting was reached. While using the 14-gauge outlet, the balloon did not pop even at the highest setting (60 calories per second). However, using the 7-French gauge, the balloon popped at 45 calories per second. After this test, the 14-gauge outlet was used for early experiments so as not to pop balloons. This continued until Experiment 2, where a more realistic outlet was used.

This outlet size was not meant to reflect the exact size of the outlet to be used clinically and was a rough approximation that later studies could fine-tune.

To determine the outlet size for Experiment 2 onwards, the outer diameters for both the inlet and pressure sensor tube were subtracted from the largest typical diameter for a catheter (18- French). Based on this information, the new outlet size could be, at most, around 2.9 millimeters. The outlet used for all future experiments was 2.6 millimeters. Because the outlet size will affect the exact treatment penetration, changing the outlet size could be an avenue for adjustment in future studies.

**Chicken Breast**

*Parameter Testing*

Several attributes of the chicken were under consideration from early in the experimental process. First, the size and shape of the chicken had to be determined. The chicken used was not made into the size and shape of the bladder. It was not feasible to cut the chicken into a thin, wall-like structure on the inside of the balloon. Therefore, several experiments were used to verify that the chosen sizes and shapes were adequate substitutes for modeling the bladder wall.

One area of concern was the size and thickness of the chicken. For Experiment 1, 30 millimeter-sided cubes were arbitrarily chosen. However, as this model would attempt to model a much thinner substance with chicken, it had to be confirmed that the difference in thickness would not prevent reasonable comparison. This was addressed in an experiment in which the penetration was compared between samples of chicken with different thicknesses. These sizes were a cubed sample of chicken with 20-millimeter sides, a cubed sample of chicken with 30-millimeter sides, and a sample of chicken kept as large as possible while still fitting within the balloon. All trials were conducted using the default settings for both inflation and treatment. This

experiment showed no considerable difference between the three different thicknesses across two trials for each thickness. After this experiment, for Experiment 2 onwards, it was decided that 20 millimeter-sided chicken cubes would be used due to the greater ease of placement and manipulation.

Another aspect of the chicken that had to be addressed was its placement. An early assumption was that because the ablation method relied on circulation, it should not matter where the chicken was placed in the balloon. However, this assumption also needed to be confirmed. This was validated through an experiment in which 20 millimeter-sided chicken cubes were placed in several distinct positions within the balloon. These included right next to the inlet, on either side, and as far as possible from the inlet. This was repeated twice for each position at the default inflation and treatment settings. This experiment showed no considerable difference between any of the positions. Moving forward, due to the ease of placement, the chicken was placed near the edge of the balloon and next to the inlet.

An additional pilot test showed no considerable differences between chicken used immediately and chicken frozen and thawed as long as the chicken was thawed entirely and brought to a consistent temperature before the treatment.

*Preparation*

All chicken for this experiment was obtained from a grocery store. The same brand and variety of chicken breast was purchased each time. The chicken was bought fresh and brought to the lab location. The chicken breast was separated and then put in the freezer for several hours to make it easier to cut consistently. It was cut into the required-sized cube once it had solidified enough to cut without compression. The cubes were frozen again if not used immediately or placed in a hot water bath at 37°C. After treatment, the chicken was removed immediately from

the balloon to prevent additional energy transfer. This would be an area that future studies might wish to adjust since residual energy might continue penetrating in an actual bladder.

**Balloon Parameters**

The balloon size was determined based on an extended or partially full bladder size. While the treatment would not be performed on a full bladder, bladders have better structural integrity than a balloon. While empty, the bladder is roughly tetrahedral, while the balloon is entirely flat in a trapezoidal shape. So, approximating a full bladder was thought to be more similar to the volume available for circulation in an actual bladder. Therefore, the goal of the balloon was to approximate the size and shape of a mostly inflated to fully inflated bladder. When unfilled and measured flat, the balloons had linear measurements of approximately 15 centimeters by 10 centimeters. When filled, they had a volume of approximately 500 mL, making it on the larger side of a potential patient's bladder size but within a reasonable and realistic bound.

Validation testing ensured the template was consistent. The balloon was sealed on two sides using a heat sealer. One side was cut open to allow the chicken to enter, after which that side was also sealed.

**Ideal Results Parameters**

Based on the limitations of the model, the ideal results for a sample of chicken had to be determined. After a discussion with a urologist from Francis Medical, the ideal depth was determined to be 1 millimeter. This depth would address carcinoma *in situ* and other Stage 0 NMI bladder cancers, and some less invasive Stage I cancers. This would stay within the transitional epithelium and, at most, reach the lamina propria. Both layers are more likely to heal without concern from an ablation injury. This was also based on Francis Medical's research into

prostate cancer. While the exact mechanism and healing process had not been determined for a bladder specifically, this would give a reasonable expectation for considering an experiment a success. Future studies with actual bladder tissue may find this more penetration than desired, but it was a useful foundational measurement.

## Specific Experimental Methods

### Experiment 1 – Device Settings and Penetration Depth and Definition

The first round of experiments aimed to develop a baseline understanding of the system and to determine the relationship between the programmable factors and the penetration depth. To determine these relationships, a two-factor, two-level experiment was developed. The default inflation was used since the primary focus was on the treatment length and energy. An outlet of 14-French gauge (or 4.62 millimeters) was used since prior proof-of-concept experiments had shown that it was the least likely of the available outlet sizes to pop the balloons at the higher energies. At this point, catheter outlet size constraints were not considered.

The chicken was cut into cubes of 30 millimeters on each side and brought to a standard 37°C (human body temperature).

*Table 2 High and low factors for Experiment 1*

| | High | Low |
|---|---|---|
| **Time (secs)** | 103 | 20 |
| **Energy (cal/sec)** | 50 | 30 |

The values that were set for this experiment are shown in Table 2. The values for inflation were the default settings. The goal was to have a measurable difference between the four values, so the extreme ends of the system's capabilities were used. However, a previous experiment proved that using the full 206-second available time would thoroughly penetrate the chicken, so the midpoint of the time was used instead. Due to the results, which will be discussed in a later

section, this time was determined to be far too long for the experiment, so subsequent experiments drastically cut down on the time for treatment. Each combination of settings was replicated, and the order of experimental runs was randomized. This meant a total of 8 experimental runs.

**Experiment 2 – Lower Time Device Settings and Penetration Depth and Definition**

The original plan for Experiment 2 had to be adjusted due to unforeseen circumstances. When prepping the delivery system, it was discovered that the gate check valve next to the syringe had broken. This development meant the steam flow was being split and had little pressure. This inconsistency caused errors in the system. Once the cause of the issues was determined, the gate check valve was replaced. However, another problem arose. Balloons were popping at energies that they previously would not have. They were popping during the inflation stage. It was determined that the gate check valve may have been marginally faulty earlier and injected slightly lower pressure than stated. Therefore, the experiment went on with the caveat that the inflation stage was reduced to 40 cal/sec for the energy, and all the treatment energies were lowered slightly. The treatment values are found in Table 3. Each setting combination was replicated for a total of 18 experimental runs. For this experiment, a sample size of twenty-millimeter-sided cubes and an outlet size of 2.6 millimeters diameter were used.

*Table 3 Setting values for Experiment 2*

| | **Low** | **Med** | **High** |
|---|---|---|---|
| **Time (secs)** | 10 | 30 | 50 |
| **Energy (cal/sec)** | 22 | 30 | 40 |

**Experiment 3 – Inflation Stage Depth Penetration**

One issue with every experiment was that all the experimental runs, including the lowest times and energies, penetrated more than the desired depth. It was theorized that the desired depth penetration might occur during the initial inflation stages.

An experimental design was developed to test this theory. The delivery system would not progress past the inflation stage in this experiment. Instead, the experiment would be abruptly cut off during the inflation stage once the balloon was visually inflated to see how much penetration had occurred before the start of the treatment phase. The pressure sensor was used to ensure that no upper threshold was passed. This process was repeated at several different energies. The energies chosen for this experiment were 25 calories per second, 35 calories per second, and 45 calories per second. These energies represented a realistic spectrum for inflation.

The experiment was run with twenty millimeter-sided cubes of chicken brought to 37°C and an outlet inner diameter of 2.6 millimeters. Each treatment energy setting was replicated once for six experimental runs.

**Experiment 4 – Air Inflation**

The water vapor needed to circulate for the ablation to be as effective as possible. In previous experiments, the chicken tended to stick to the foil of the mylar balloon, which potentially affected the steam's ability to circulate effectively. In addition, when not full, a bladder maintains some shape integrity, which the balloon does not.

To combat both concerns, an experiment was created in which the balloon was inflated with air before the water vapor inflation. The chicken cubes were all twenty millimeters on each side, and the experiment was run at 40 calories per second for 10 seconds. This experiment aimed primarily to determine if there was a considerable difference between experiments if the

balloon already had some pressure and shape. As this was essentially a pilot test, only four total runs were used, two air-inflated samples and two regular inflations, to ensure that there would not be any considerable difference between an already-inflated balloon and an uninflated balloon.

**Experiment 5 – Ideal Depth**

This final experiment was to see if, considering all the information gathered, a sample of chicken could be penetrated to a 1-millimeter depth. This was performed using the 2.6 millimeter outlet and 20 millimeter-sided cubes of chicken. The inflation was set to 50 calories per second, and the time was six seconds. Four trials were conducted at the same settings and the depth penetration and definition were recorded.

Another sample of chicken with treatment energy set to 45 calories per second was circulated. However, the depth penetration was insufficient to collect meaningful data.

**RESULTS**

**Experiment 1 – Device Settings and Penetration Depth and Definition**

Experiment 1 was run at the energies and times noted above. The main results from this initial experiment were the depth penetration and the definition scores. The raw measurements are shown in Table 4. This data consolidates the measurements from each of the three sides of each sample of chicken into one mean measurement.

*Table 4 Raw data for depth penetration and definition score from Experiment 1*

| Energy | Time | Depth Penetration (mm) | Definition |
|---|---|---|---|
| High | High | 6.98 | 6 |
| High | High | 11.27 | 4 |
| **High-High Mean** | | 9.13 | 5 |
| High | Low | 3.72 | 9 |
| High | Low | 3.24 | 7 |
| **High-Low Mean** | | 3.48 | 8 |
| Low | High | 12.38 | 2 |
| Low | High | 5.86 | 5 |
| **Low-High Mean** | | 9.12 | 4 |
| Low | Low | 3.56 | 8 |
| Low | Low | 3.21 | 10 |
| **Low-Low Mean** | | 3.39 | 9 |

Table 5 simplifies the depth penetration data and shows the mean in millimeters for each factor combination.

*Table 5 Depth penetration for Experiment 1*

| Energy | Time | Mean (mm) |
|---|---|---|
| High | High | 9.13 |
| High | Low | 3.48 |
| Low | High | 9.12 |
| Low | Low | 3.39 |

The relationship between the various factors and depth penetration is demonstrated in Table 6. This table shows the depth penetration for each experimental factor's setting. The highest mean penetration is for high times, and the lowest is for low times. The mean resulting from each time setting is visible across each row. The mean depth penetration for each energy setting is visible by looking vertically down a column. The final mean in the rightmost bottom corner is the overall mean across the entire experiment which was 6.28 millimeters. The difference between the means for low and high time is a drastic, positive difference. When

looking at the difference in mean across the energies, there is only a very slight positive increase when the energy goes from low to high.

*Table 6 Depth penetration in millimeters*

| | | Energy | | |
|---|---|---|---|---|
| | | Low | High | **Mean** |
| **Time** | Low | 3.39 | 3.48 | **3.43** |
| | High | 9.12 | 9.13 | **9.12** |
| | **Mean** | **6.25** | **6.30** | **6.28** |

Figure 11 also demonstrates these same relationships. This figure shows a representation of depth penetration in millimeters across two different times and at two different energy settings, notated in blue and red.

Since the lines are so visually similar, the corresponding linear equations of best fit are also included. Note that the lines of the two different energy settings are nearly on top of each other. There is little difference between the two energy settings' depth penetration.

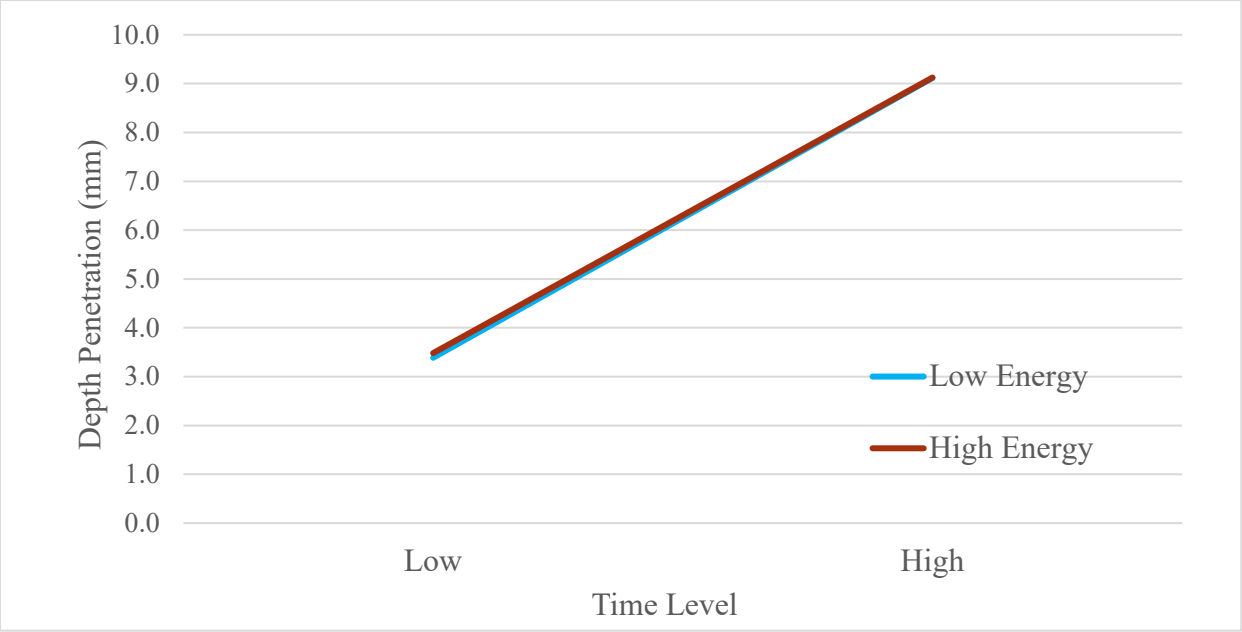


*Figure 11 Depth penetration comparison for Experiment 1*

The relationship between the various factors and the definition score is shown in Table 7. The definition is a score from 1 to 10, with 10 being the most obvious delineation between the treated and non-treated chicken. The highest score is at low time, while the lowest is at high time.

*Table 7 Definition score means for the experimental factors.*

| | | **Energy** | | |
|---|---|---|---|---|
| | | Low | High | **Mean** |
| **Time** | Low | 9 | 8 | **8.5** |
| | High | 3.5 | 5 | **4.25** |
| | **Mean** | **6.25** | **6.5** | **6.375** |

This data is visualized in Figure 12. This figure shows the low and high time across the bottom with low energy as the blue bars and the orange bars as high energy. Both the low and high energy bars at low time are considerably higher than either bar from the high time. At the low time, there is not much of a difference between low and high energy, and low energy has a slightly high definition. At the high time, the low energy has a lower definition, and again, there

is not much difference between the two bars. When both energy and time were low, the highest definition was achieved with a score of 9.

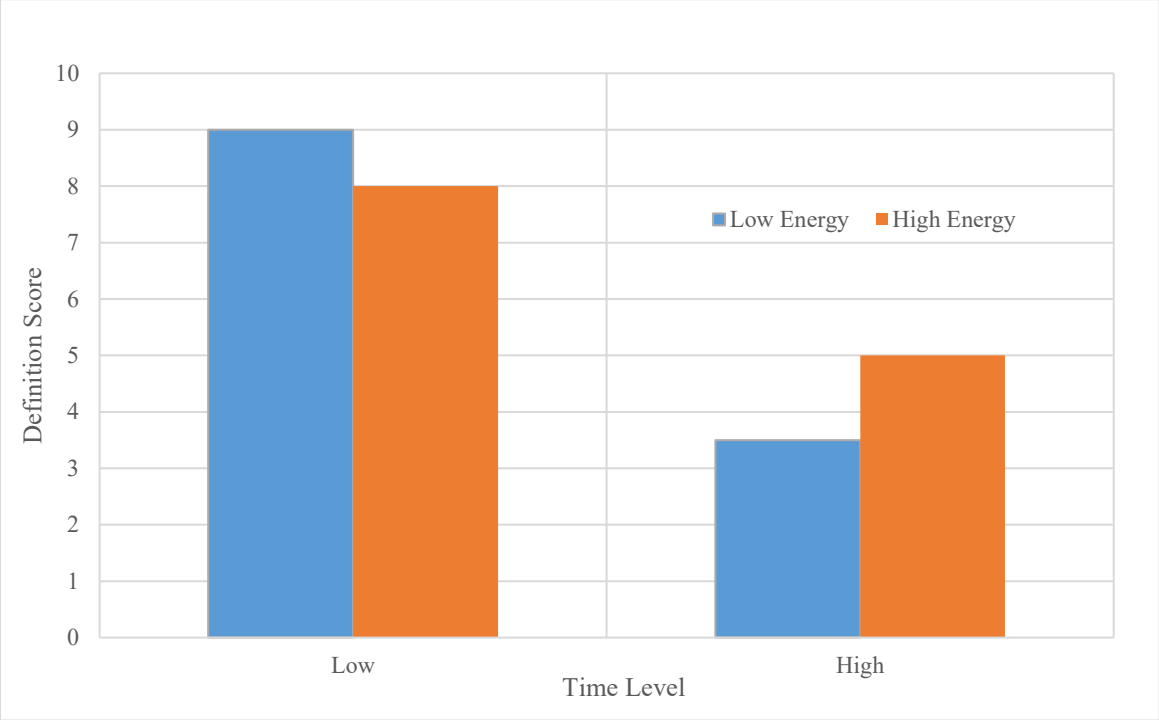


*Figure 12 Definition score comparison for Experiment 1*

## Experiment 2 – Lower Time Device Settings and Penetration Depth and Definition

Experiment 2 had a similar data set but included an additional midpoint for each factor. The raw data for all experimental runs is in Table 8. This table contains the definition scores, penetration in millimeters, and the means for each factor combination.

*Table 8 Raw data from Experiment 2: Depth penetration and definition scores*

| Energy | Time | Definition | Penetration (mm) |
|---|---|---|---|
| Low | Low | 7 | 4.34 |
| Low | Low | 4 | 5.94 |
| **Low-Low Mean** | | **5.5** | **5.14** |
| Low | Med | 8 | 2.44 |
| Low | Med | 5.5 | 4.15 |
| **Low-Med Mean** | | **6.75** | **3.29** |
| Low | High | 6.5 | 3.28 |
| Low | High | 6.5 | 4.09 |
| **Low-High Mean** | | **6.5** | **3.68** |
| Med | Low | 7.5 | 4.30 |
| Med | Low | 7.5 | 4.63 |
| **Med-Low Mean** | | **7.5** | **4.47** |
| Med | Med | 4.5 | 5.13 |
| Med | Med | 5 | 4.72 |
| **Med-Med Mean** | | **4.75** | **4.93** |
| Med | High | 7 | 4.07 |
| Med | High | 6 | 4.56 |
| **Med-High Mean** | | **6.5** | **4.32** |
| High | Low | 3.5 | 5.64 |
| High | Low | 4 | 6.44 |
| **High-Low Mean** | | **3.75** | **6.04** |
| High | Med | 5 | 5.48 |
| High | Med | 6 | 3.82 |
| **High-Med Mean** | | **5.5** | **4.65** |
| High | High | 3 | 4.58 |
| High | High | 4 | 4.95 |
| **High-High Mean** | | **3.5** | **4.76** |

This experiment had a lower maximum time, so the depth penetration was lower across the entire experiment. However, none of the experimental runs achieved the ideal 1 millimeter

depth penetration. The differences between the different runs were not as dramatic as those between the previous experimental runs. Table 9 contains a simplified version of the raw data, containing only the mean for each factor combination.

*Table 9 Depth penetration for Experiment 2*

| Energy | Time | Mean (mm) |
|---|---|---|
| Low | Low | 5.14 |
| Low | Med | 3.29 |
| Low | High | 3.68 |
| Med | Low | 4.47 |
| Med | Med | 4.93 |
| Med | High | 4.32 |
| High | Low | 6.04 |
| High | Med | 4.65 |
| High | High | 4.76 |

The relational data for depth penetration in Table 10 shows the means across several factors. The mean depth penetrations for this experimental run were between 4 millimeters and 5.5 millimeters.

*Table 10 Relationships between factors for depth penetration*

| | | **Energy** | | | |
|---|---|---|---|---|---|
| | | Low | Med | High | **Mean** |
| **Time** | Low | 5.14 | 4.47 | 6.04 | **5.21** |
| | Med | 3.29 | 4.93 | 4.65 | **4.29** |
| | High | 3.68 | 4.32 | 4.76 | **4.25** |
| | **Mean** | **4.04** | **4.57** | **5.15** | **4.59** |

This data is visualized in Figure 13. The most depth penetration occurred in experimental runs with high energy and low time, with a mean of 6.04 millimeters. The least depth penetration was 3.29 millimeters and occurred at low energy and medium time.

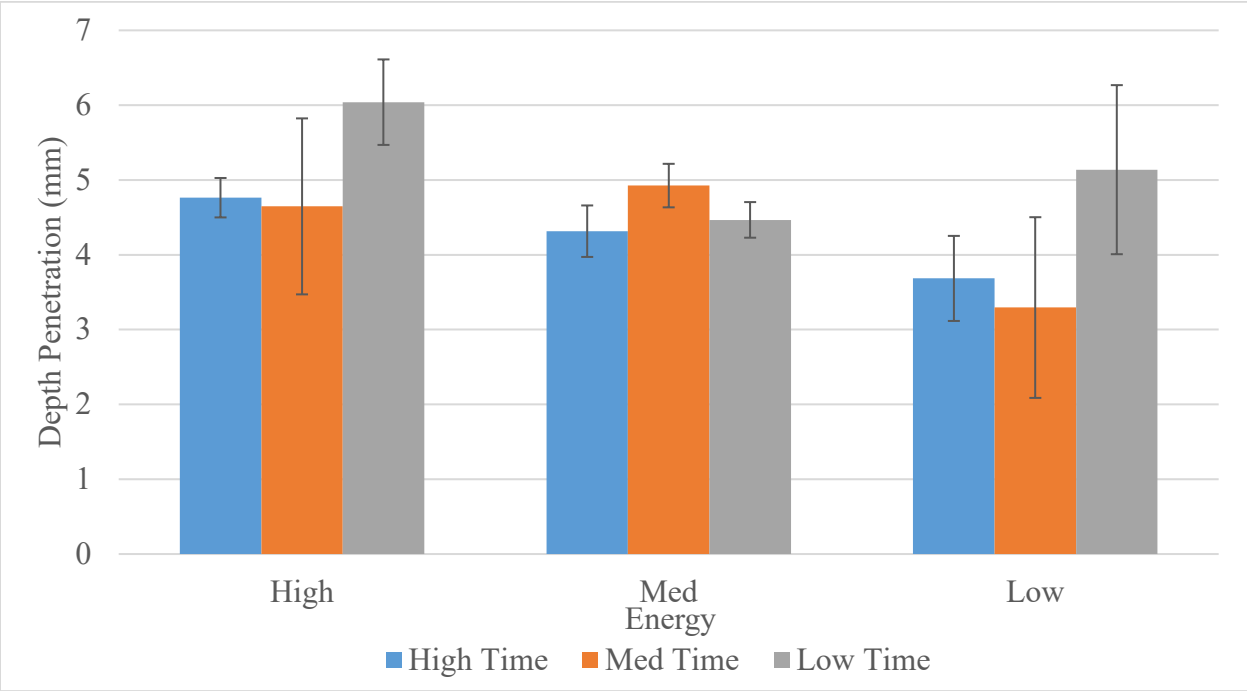


*Figure 13 Depth penetration for Experiment 2*

The definition score data is simplified in Table 11. The highest definition scores in this set of experiments were for medium energy and low time runs, with an average score of 6.75. The lowest definition scores were for high-time and high-energy runs.

*Table 11 Simplified definition scores for Experiment 2*

| Energy | Time | Definition Score |
|---|---|---|
| Low | Low | 5.5 |
| Low | Med | 7.5 |
| Low | High | 3.75 |
| Med | Low | 6.75 |
| Med | Med | 4.75 |
| Med | High | 5.5 |
| High | Low | 6.5 |
| High | Med | 6.5 |
| High | High | 3.5 |

The factor relationship data for definition scores is in Table 12. This table shows the mean score across several factors. The range for these definition scores was much smaller than in the previous experiment.

*Table 12 Relational means across factors for definition scores*

| | | Energy | | | |
|---|---|---|---|---|---|
| | | Low | Med | High | **Mean** |
| **Time** | Low | 5.50 | 6.75 | 6.50 | **6.25** |
| | Med | 7.50 | 4.75 | 6.50 | **6.25** |
| | High | 3.75 | 5.50 | 3.50 | **4.25** |
| | **Mean** | **5.58** | **5.67** | **5.50** | **5.40** |

This data is visualized in Figure 14. The visualization makes it easier to see a trend across a factor.

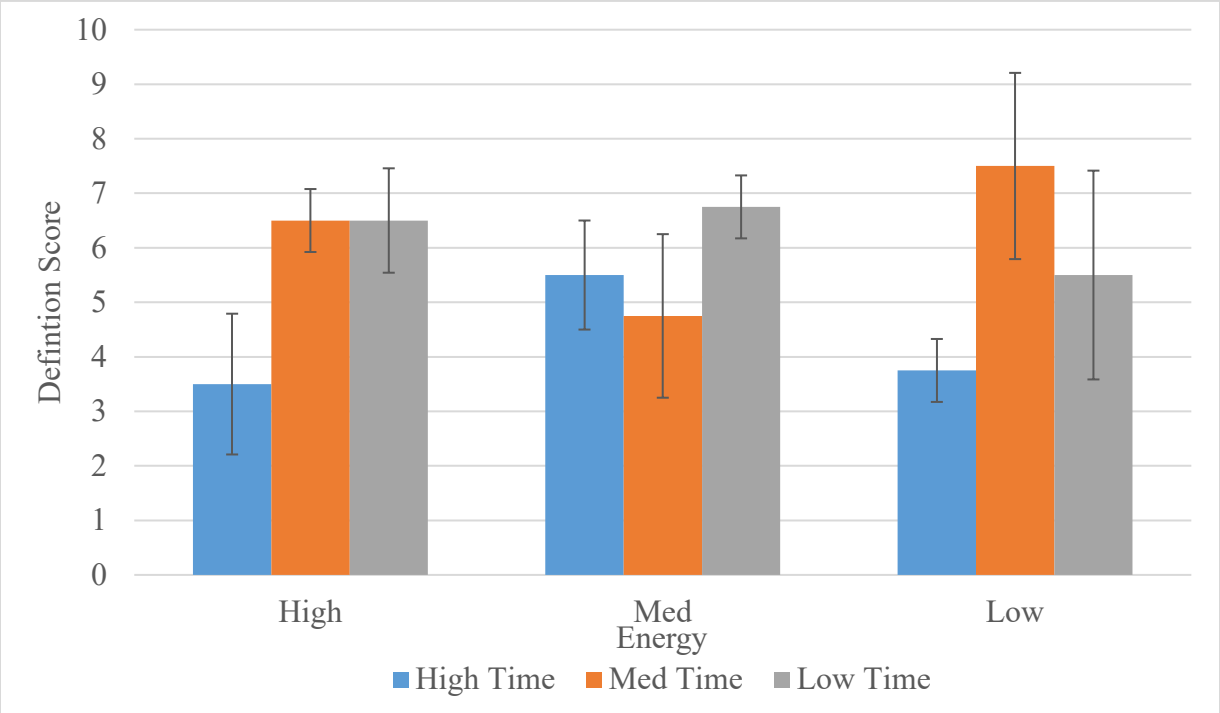


*Figure 14 Definition scores for Experiment 2*

For instance, the lowest definition was always in experiments with high time, and the next closest to the lowest definitions was in medium-time experiments. The higher-definition

experiments were all either low or medium-time experiments. There is less evidence of a trend across energies.

**Experiment 3 – Inflation Stage Depth Penetration**

There were a couple of critical data sets in this round of experiments. One of the first essential areas was the time needed to reach total inflation. The time required was drastically different across the three inflation energies tested. These are shown in Table 13. Predictably, the experiments with the highest inflation energy had the fastest inflation, with an average time of 9.5 seconds. The lowest time was for experiments using an energy level of 25 calories per second.

*Table 13 Mean times needed for inflation*

| | **Inflation Energy (cal/sec)** | | |
|---|---|---|---|
| | 25 | 35 | 45 |
| **Mean Time (sec)** | 23.25 | 14.1 | 9.5 |

In one case, shown in Table 14, the inflation took over 30 seconds. The most consistent times were 35 calories per second experiments, which took an average of 14.1 seconds to inflate. Table 14 also contains the depth penetration, and it is essential to note that, yet again, all the experiments were more than the 1-millimeter ideal depth. However, it is much closer to the optimal depth penetration.

*Table 14 Depth penetrations at inflation point for different energies.*

| | Inflation Energy (cal/sec) | | | | | |
|---|---|---|---|---|---|---|
| | 25 | | 35 | | 45 | |
| **Trial #** | 1.00 | 2.00 | 1.00 | 2.00 | 1.00 | 2.00 |
| **Time Needed (sec)** | 30.10 | 16.40 | 14.20 | 14.00 | 8.30 | 10.70 |
| **Depth (mm)** | 2.39 | 1.80 | 2.48 | 3.23 | 1.94 | 1.82 |
| **Mean Depth (mm)** | 2.10 | | 2.86 | | 1.88 | |

Interestingly, as shown in Figure 15, the depth penetration was highest for the middle energy. The lowest mean depth penetration was 1.56 millimeters in the 45 calories per second experiments.

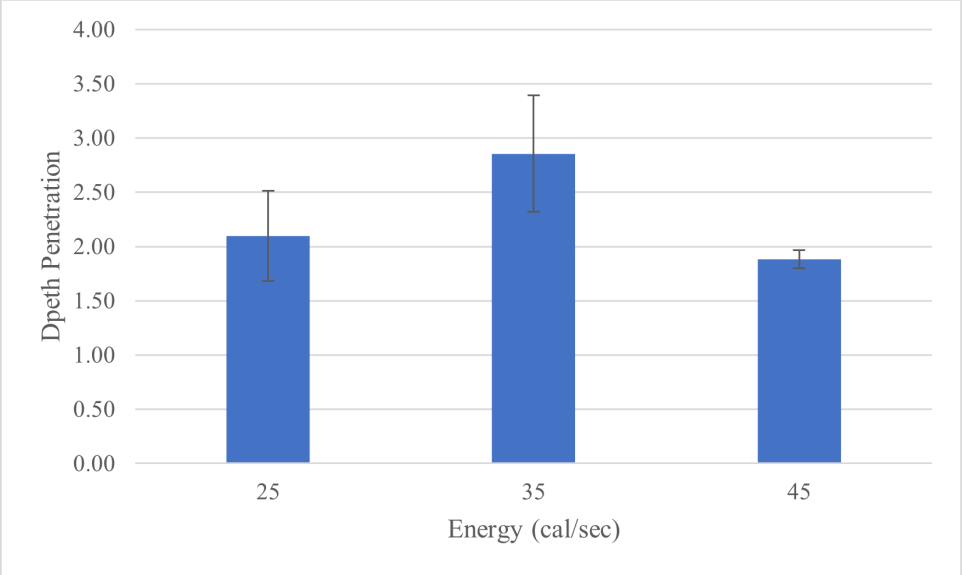


*Figure 15 Depth penetration for variable energies*

## Experiment 4 – Air Inflation

Experiment 4 required less data analysis and had a much smaller dataset. The primary data was the depth penetration, and no data regarding the definition was collected. This is shown in Table 15.

*Table 15 Means for Experiment 4*

| Trial | Depth Penetration (mm) |
|---|---|
| 1 | 2.33 |
| 2 | 1.73 |
| Mean | 2.03 |

The most important feature of this experiment was that the depth penetration was consistent with results from previous experiments.

## Experiment 5 – Ideal Depth

Experiment 5 had a similar, smaller dataset to Experiment 4. There were only four trials. The overall mean depth penetration for these four trials was 1.04 millimeters, and the definition averaged a score of 8. A sample of chicken treated for 5 seconds was also attempted during this experiment. However, it did not penetrate enough for data to be collected.

The raw data from the experiment is shown below in Table 16.

*Table 16 Depth penetration and definition scores for Experiment 5*

| Trial | Depth Penetration (mm) | Definition Score |
|---|---|---|
| **1** | 1.16 | 9 |
| **2** | 0.93 | 8 |
| **3** | 0.95 | 7 |
| **4** | 1.13 | 8 |
| **Mean** | 1.04 | 8.5 |

This experiment also had some of the best-defined edges where the depth of the thermal energy transfer could be clearly seen. Several of these well-defined edges are visible in Figure 16.

*Figure 16 Close-up to show a defined edge on a sample of chicken*

Another important note was that the balloons did inflate, although just barely. This inflation means that the water vapor was able to circulate.

## DISCUSSION

### Experiment 1 – Device Settings and Penetration Depth and Definition

The first thing that needed to be determined was the relationship between time and energy, the definition scores, and depth penetration. This would guide future experiments and help determine whether there was considerable interaction between time and energy or if they would interact with depth penetration and definition separately.

As evident from Table 4, all experiments' depth penetrations were over the optimal 1-millimeter depth. Table 5 also highlights that with depths of nearly nine times the ideal depth, the high times were far too large.

The most illustrative table is Table 6. This table compares the mean penetration across any factor. The difference between high and low times is especially evident here. As the difference between energy levels was not as drastic, it is apparent that time has a more considerable overall effect on the depth penetration. The difference between the means for low and high time is a drastic, positive difference. Looking at the difference in mean across the energies there is only a very slight positive increase when the energy goes from low to high energy.

When this type of information is shown graphically, it shows interactions between factors. If there is minimal interaction, the lines will be parallel. If there is considerable non-linear interaction, the two lines would intersect dramatically. Figure 11 has similar lines for high and low energy across low and high times. This similarity means that there is little non-linear interaction between the two factors.

Both factors will increase the depth penetration linearly regardless of the other factor's level. However, this conclusion might lack nuance due to the magnitude of the difference in the time factor. Because the differences between the factors were so substantial, it was difficult to determine the differences due strictly to the energy level for depth penetration. However, this made it obvious that time would be the main factor to investigate for depth penetration.

In Table 7, the relationships between the definition and the factors follow a similar trend to the depth penetration. The difference between the means for the two energy levels is slight. However, the difference between the means between the two levels for time was quite drastic.

In Figure 12, there is clearly a large range between the two different time levels' definition scores. Meanwhile, the energy trend was not as clear. Low time and low energy had the highest definition, with low time and high energy coming in closely behind.

This experiment highlighted that lower times would be needed for the best results.

**Experiment 2 – Lower Time Device Settings and Penetration Depth and Definition**

The intent for Experiment 2 was to validate the results of Experiment 1, but with lower times and so have a more precise indication of the relationship and interactions, if any, between the factors. Therefore, one of the first essential items of note in this experiment is the considerable lowering of the times, which impacted the overall scores and depth penetration. In addition, this round of experiments had a lower inflation energy, which also affected the data.

The range for this experiment's results was much lower for both datasets. However, the depth penetration was still six times higher than the ideal level. Future experiments must investigate inflation because even the shortest time exceeded the required penetration.

In Table 10, the mean of the different factors was much more similar than that of the previous experiments. The different energy levels had a difference in mean of only 1.11 millimeters, while the range for the various times' means was only 0.96 millimeters. This is because the range for the settings was much lower and closer together.

Figure 13 shows that there is not an especially clear trend in this case. While high energy tends to have greater depth penetration, this is not always true. This experiment also seemed to show a negative correlation with time. This was never replicated in other experiments. But in Figure 13, there is a slight downward trend as time increases. This unexpected result could be explained by multiple reasons. Minor inconsistencies with the experimental design could easily result in a noticeable difference. In addition, these sample sizes were quite small, making it difficult to determine when there were outliers. This would be an area that would merit further investigation to ensure the validity of other experiments.

Based on this data, the chicken had already undergone considerable depth penetration when the treatment period started. The inflation length must be much shorter to attain the ideal depth penetration.

The definition scores for this round of experiments were lower than for Experiment 1, with the highest score being only 7.5. This could be explained by the differences in the gate check valve after it was replaced. From Table 11, there is a considerable difference in the definition when the time is high. The mean across all experiments with a high time has the lowest scores for definition. Low and medium times do not have as big of a difference,

supporting the idea that most of the penetration occurs at the inflation stage and that only considerably increasing the time affects the depth penetration and definition.

The significance of the high time is evident in Figure 14 as well. This figure clearly shows that while there are few concrete trends, definition suffers when the time is high. The blue bars that represent runs with high times have the least definition.

**Experiment 3 – Inflation Stage Depth Penetration**

Using variable inflation energies and seeing the length of inflation was valuable because it framed previous experiments and guided the final sets of experiments. One of the most valuable things to come out of this experiment was based on the times needed for inflation. The fastest times from the highest energy experiments were the only experiments inflated in under 10 seconds. Note that this time was the default for inflation in previous experiments, so based on this experimental data, only the experiments with a 45-calorie per second or higher inflation energy had attained total inflation during the inflation period.

As mentioned in the experimental section, this experiment also revealed that the balloon model might have limitations related to the chicken sticking to the mylar, making it more difficult for the water vapor to circulate.

The results of this experiment also made it very clear that most of the energy transfer occurs within a much shorter timeframe than initially thought. Even the shortest times resulted in more depth penetration than was desired. This potentially poses an issue. The purpose of using this variety of water vapor ablation is that it utilizes convective heating rather than conductive. If the bladder is not inflated before the water vapor circulates, it may have uneven energy transfer. This could be mitigated by using a different outlet size, but the size of a catheter also constrains the size of the outlet.

The depth penetration of these experiments is also notable for how much closer to the optimal depth they are. The mean depth was highest for the middle energy, which was surprising. However, this makes sense when considering that the higher energy had a considerably lower amount of time, and the lower energy had a higher energy for a similar amount of time. Trial 2 for 25 calories per second had a comparable time to inflation to both trials for 35 calories per second, but lower energy resulted in considerably lower depth penetration.

Based on these factors, a 45-calorie-per-second inflation energy would be preferable as it has the closest depth penetration to optimal and the lowest time to inflation.

**Experiment 4 – Air Inflation**

The primary goal of this experiment was to determine if there was a considerable difference in depth penetration if the bladder was already partially inflated. Less data was required for this experiment. In two trials at 45 calories per second for 10 seconds, the overall mean was 2.03 millimeters. This is consistent with the results of the previous experiment and infers that the difference between a partially inflated balloon and a fully inflated balloon is not considerable.

**Experiment 5 – Ideal Depth**

This experiment aimed to use previous experiments' data and get two well-defined replicants close to the ideal depth penetration. Considering the limitations of this model, this was a successful experiment. While the data was not as consistent as desired, the overall mean was trending toward the ideal depth penetration. In addition, the clarity and definition of the edges was very encouraging. Based on the lack of usable results from the chicken treated for five seconds, lowering the time to get a smaller depth penetration would negatively affect the

uniformity. If portions of the surface are entirely untreated, one of the primary benefits, the ability to address all cancer on the surface at once, is defunct.

Overall, this was the most promising result of the experiments. However, the brevity of the treatment length could make it challenging to apply in real life.

## CONCLUSION

The findings in this thesis are preliminary, but they are encouraging. Using a balloon model, this project aimed to determine the viability and dosimetry for a water vapor ablation treatment for bladder cancer cells. The objective was to provide a framework and baseline for future research. Specifically, the goal was to determine relationships between definition, depth penetration, and treatment settings. Because a model was being used rather than an actual bladder, inherent limitations must be noted and addressed for future research.

The relationship between the depth penetration and the treatment settings was quite different than initially expected. Specifically, there was less interaction between time and energy than anticipated. While time and energy positively correlated with depth penetration, time was much more influential in determining the final depth penetration. Even more surprising, depth penetration was not substantially changed by the energy level and instead was much more dependent on how long the treatment was.

In the case of the definition, time was again the most considerable determinant, and surprisingly, energy did not have much impact on it. As the time increased, the definition decreased, and changing the energy did not make a difference at the tested treatment times.

These experiments concluded that adjusting the time was the best way to determine the result. While energy had some influence on the depth penetration, changing the time drastically was much more likely to influence these results. Future experiments could investigate whether

energy could be used to fine-tune the results while major differences would be controlled via time.

While these conclusions are helpful, much more information could be gained through future endeavors. Initial plans were to include an experiment using a pig bladder. Unfortunately, difficulties in obtaining a fresh pig bladder prevented this experiment. For future research, this experiment would be beneficial in determining how relevant this model is. After determining the depth penetration and definition in a pig bladder, experimental runs with the model at the same settings could generate a predictive model showing the relationship, if any, between the penetration of chicken and the penetration of a pig bladder wall.

This model had many limitations. One of the most important is that the labor-intensive nature of these experiments makes it difficult to have a larger sample size. With a larger sample size, it would be easier to determine the exact nature of interactions between factors and note trends. In addition, outliers would be more easily identified.

Energy and water vapor flow inconsistencies could be attributed to mechanical difficulties with the gate check valve. This was addressed but could have had lingering impacts on early experiments. Other potential issues that may have affected the experiments to varying degrees include the balloons not sealing correctly, the chicken sticking to the mylar, and the possible presence of remnant fluids from the chicken defrosting. The balloon did not have the exact shape and size of the bladder. Custom mylar ballons would be a good investment for future experiments. It was also challenging to ensure that the chicken did not cool to room temperature while sealing the balloon and setting up the rest of the experiment. It would be beneficial to see how different the results would be if the entire balloon were in a water bath at 37°C. Finally, chicken tends to lose its shape when raw, meaning that while frozen, there might be discrete

sides that face out; when defrosted, the sides are less apparent. Many of these limitations could be addressed or found inconsequential in future experiments with this model. Another limitation that could be addressed is the lack of recording for the pressure sensor. Continuous data for the pressure was not collected. Instead, current pressure during the experiments was displayed, and any high pressures were noted.

Another very important limitation was the lack of bladder tissue. The bladder has distinct layers which are made of different types of tissue. The chicken breast does not contain transitional epithelium cells and is primarily comprised of muscular tissue. The structure of the transitional epithelium cells and the fact that they change shape when stretched are essential factors that could affect how well water vapor can infiltrate and surround this top layer. In addition, cells from the chicken are dead while the cells that would be treated with this method are still alive. Living cells could respond very differently than the dead cells tested. These transitional epithelium cells also will not have the same expansion characteristics as a balloon.

This set of experiments was a solid foundation, but much more research is needed to fully determine the dosimetry of a human bladder. These experiments showed that water vapor ablation does occur when water vapor is injected into a chamber through a catheter. In addition, it showed that this ablation, with the right dosimetry, can effectively and uniformly ablate a minimal distance into a tissue. Whether the treatment time and energy discovered here apply to real-life ablation remains to be seen. However, water vapor ablation for bladder cancer cells is a worthwhile strategy to continue investigating.

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**APPENDIX**

**Table showing the relationship between voltage output and pressure**

| **Output (V)** | **0.10** | **0.20** | **0.30** | **0.40** | **0.50** | **0.60** | **0.70** | **0.80** | **0.90** | **1.00** |
|---|---|---|---|---|---|---|---|---|---|---|
| **P (psi)** | 14.60 | 14.64 | 14.68 | 14.71 | 14.75 | 14.79 | 14.83 | 14.87 | 14.91 | 14.95 |
| **Rel P (psi)** | -0.09 | -0.05 | -0.01 | 0.03 | 0.07 | 0.11 | 0.14 | 0.18 | 0.22 | 0.26 |
| | | | | | | | | | | |
| **Output (V)** | **1.10** | **1.20** | **1.30** | **1.40** | **1.50** | **1.60** | **1.70** | **1.80** | **1.90** | **2.00** |
| **P (psi)** | 14.98 | 15.02 | 15.06 | 15.10 | 15.14 | 15.18 | 15.21 | 15.25 | 15.29 | 15.33 |
| **Rel P (psi)** | 0.30 | 0.34 | 0.38 | 0.41 | 0.45 | 0.49 | 0.53 | 0.57 | 0.61 | 0.64 |
| | | | | | | | | | | |
| **Output (V)** | **2.10** | **2.20** | **2.30** | **2.40** | **2.50** | **2.60** | **2.70** | **2.80** | **2.90** | **3.00** |
| **P (psi)** | 15.37 | 15.41 | 15.45 | 15.48 | 15.52 | 15.56 | 15.60 | 15.64 | 15.68 | 15.71 |
| **Rel P (psi)** | 0.68 | 0.72 | 0.76 | 0.80 | 0.84 | 0.88 | 0.91 | 0.95 | 0.99 | 1.03 |