



Comparison of the tissue interaction between thermal ablation and cryotherapy as treatment for cervical precancerous lesions in an ex-vivo model

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Abstract

Both cryotherapy and thermal ablation are treatment methods for cervical precancerous lesions in screening programs in resource constrained settings. However, for thermal ablation the World Health Organization stated that there is insufficient data to define a standard treatment protocol. This study used an ex-vivo model to compare the tissue interaction of both cryotherapy and thermal ablation to contribute to a treatment protocol. We used porcine tissue to measure the temperature profile over time at 0, 2, 4 and 6 mm depth. For cryotherapy the standard double freeze method was used, thermal ablation was applied for one cycle of 60 s with 100 °C. Based on literature search we used 4 mm depth as landmark for the depth of precancerous lesions, and -10 °C for cryotherapy and 46 °C for thermal ablation as critical temperature to induce cell necrosis. Cryotherapy achieved the critical temperature for tissue necrosis (-10 °C) in 3 out of 6 experiments at 4 mm depth, median minimum temperature was -9.6 °C (IQR 25–75 -15.8 °C to -4.9 °C). Thermal ablation achieved the critical temperature for tissue necrosis (46 °C) in 3 out of 7 experiments at 4 mm depth, median maximum temperature was 43.1 °C (IQR 25–75 42.3 °C to 49.9 °C). Both treatment modalities achieved tissue necrosis at 4 mm depth in our ex-vivo model. For cryotherapy the double freeze technique should be used. For thermal ablation a single application less than 60 s might not be sufficient and multiple applications should be considered.

Keywords Thermal ablation · Cryotherapy · Ex-vivo model · Prevention · Cervical intra-epithelial neoplasia

1 Introduction

Cervical cancer affects more than half a million women annually and the incidence is expected to increase. Women in low- and middle-income countries (LMICs) account for 85% of the

total diagnosed cases, which shows that they are disproportionately affected [1, 2]. Cervical cancer is caused by the human papillomavirus (HPV) which is transmitted via sexual contact or genital skin to skin contact [3]. The cervix is one of the primary targets of HPV infection. In more than 95% of the cases the HPV infection is contained and eliminated by the immune system. In less than 5% of the cases, HPV infection of the cervix persists which could lead to precancerous lesions, also known as cervical intra-epithelial neoplasia (CIN), and if left untreated progress to cervical cancer. The time between HPV infection and cervical cancer is 10 to 15 years, which provides a window of opportunity for screening and timely treatment of precancerous lesions [3]. With the introduction of screening programs, the incidence of cervical cancer has fallen dramatically in high income countries [4–6]. An essential part of cervical cancer screening programs is timely treatment of CIN lesions.

Cervical cancer screening programs in resource constrained settings are challenged by the limited availability of effective treatment for precancerous cervical lesions [7].

Key message In an ex-vivo model, cryotherapy and thermal ablation achieved tissue necrosis at 4 mm depth. For treatment of CIN lesions with thermal ablation a single application of less than 60 s might not be sufficient, multiple treatment cycles should be considered.

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The World Health Organization (WHO) recommends cryotherapy or thermal ablation in resource constrained settings, which can be provided in the same screening visit to prevent loss to follow-up [8, 9]. Cryotherapy uses nitrogen oxide (NO) or carbon dioxide (CO₂) gas to freeze the cells of the transformation zone of the cervix. However, practical challenges like unavailability of gas, heavy gas cylinders and fragile cryotherapy equipment due to the high-pressure system hamper broad large-scale implementation of cryotherapy in LMICs [7]. An alternative treatment method is thermal ablation, using minimal electricity to heat the cells of the transformation zone of the cervix [9]. Recent handheld models with chargeable battery have overcome the need for direct availability of electricity as a barrier to implementation in LMICs [7]. Both cryotherapy and thermal ablation can be performed by trained nurses and midwives without the need for local anaesthesia.

Local governments and non-governmental organisations have attempted to increase the number of screen-and-treat programs across LMICs and introduced thermal ablation in their screening programs. For cryotherapy a standard treatment protocol is endorsed by the WHO, however, for thermal ablation a standardized treatment protocol is not yet in place due to lack of data comparing both treatment modalities and comparing different treatment procedures for thermal ablation [9].

Previous studies showed that cure rates of CIN lesions in patients in LMICs do not differ significantly between cryotherapy and thermal ablation, demonstrating that both treatment modalities are effective [10–13]. However, there is no consensus on the treatment protocol for thermal ablation with temperatures ranging from 100 to 120 °C, duration of treatment from 20 to 60 s and the number of treatment cycles from single to repeated application ranging from 2 to 5 cycles. It is difficult to compare the different protocols in a prospective study with comparable patient cohorts, because the expected differences in effectiveness are small and significant side effects of extensive application like cervical stenosis are rare [9, 12].

In light of the lack of clinical data, this study aims to compare in an ex-vivo model the tissue interaction and temperature profile of both thermal ablation and cryotherapy to contribute to a treatment protocol.

2 Materials and methods

To compare the tissue interaction of both cryotherapy and thermal ablation we assessed the following parameters in an ex-vivo model:

1. The minimum temperature reached at the surface and at a depth of 2, 4 and 6 mm by application of cryotherapy with standard double freeze method.

2. The maximum temperature reached at the surface and at a depth of 2, 4 and 6 mm by application of thermal ablation with 100 °C for 60 s.

We selected an ex-vivo model because it enables measurements of the temperature profile and stability over time at different tissue depths for both treatment modalities with the same method.

2.1 Depth of tissue necrosis

According to the theory of Hoffman and Bischof and research of Yiu et al. adequate tissue necrosis will be achieved with cryotherapy by a minimum tissue temperature of minus 10 °C [14, 15]. The maximum tissue temperature needed to achieve tissue necrosis with thermal ablation is based on research by Brace et al., stating that irreversible cell damage begins to occur at a temperature of 46 °C [16]. A clinical study published in 1982 by Abdul Karim et al. concluded that a depth of 3.5 mm is needed to cure 95% of CIN2 and CIN3 lesions, a depth of 4.8 mm will cure 99% of CIN3 lesions [17]. In 1990 Boonstra et al. found that the depth of crypt involvement in CIN3 lesions does not extend beyond 3.6 mm, concluding that 4 mm depth of necrosis is sufficient to achieve cure [18].

2.2 Ex-vivo model

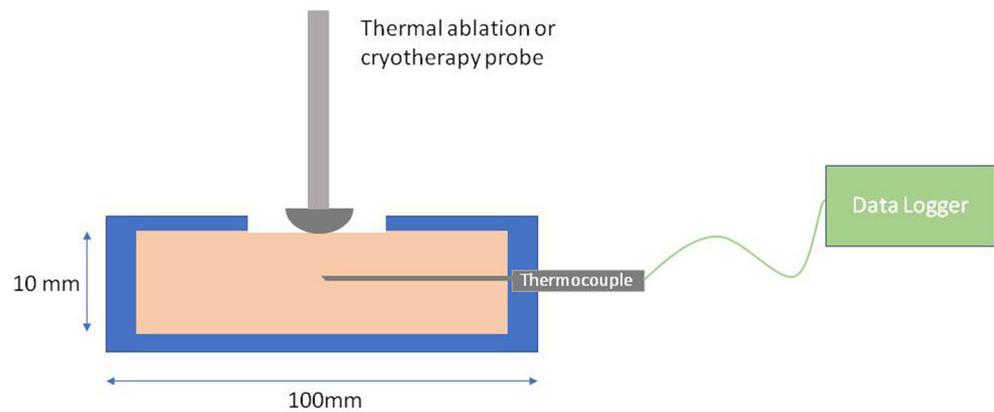
For the ex-vivo model we used porcine tissue. Porcine tissue has been used in other ex-vivo studies and the characteristics of other materials like gelatine and tofu made it unsuitable for measurements after exposure to heat and cold.

Fifteen minutes before each experiment we submerged porcine tissue (10x10x1 cm) in a plastic bag for 20 min in a water bath up to 30 °C to mimic body temperature. At a temperature of 37 °C fluid was leaking from the porcine tissue causing the tissue to dry up, therefore we used 30 °C.

We placed the preheated tissue inside a 3D printed container (printing material PLA) which housed four removable thermocouples, as illustrated in Fig. 1; one on the surface of the sample and one at 2, 4 and 6 mm distance from the surface. The surface thermocouple with type K junction provided information on the actual temperature of the tip of the device. The three other thermocouples were inserted through holes with a shape locking design to ensure positioning in the middle of the sample. All thermocouples were calibrated at room temperature, had a specific error limit of 0.3 °C and could measure temperatures up to 200 °C.

After piloting the model, we planned 7 experiments of each treatment modality. During application of cryotherapy the measurements were started 15 s before until 120 s after the treatment. For thermal ablation the measurements were started 15 s before until 60 s after the treatment.

Fig. 1 Illustration of the ex-vivo model. We used 4 thermocouples at 0, 2, 4 and 6 mm distance from the surface



2.3 Treatment

We applied cryotherapy with the Wallach WA2000 console with LL100 setup and a medical grade CO₂ tank (water volume 10 l, initial pressure 60 bar). This system included a pressure gauge, a digital temperature indicator and a timer. The CO₂ tank was replaced if the pressure indicator showed pressure in the tank was below 40 bar. We used the recommended treatment protocol for cryotherapy involving 3 min freezing, 5 min thawing, and an additional 3 min freezing, also known as the double freeze method.

For thermal ablation we used the WISAP C3 thermal coagulator powered by 230 V power socket. We applied the probe at 100 °C for 60s during one cycle, to enable analysis of the temperature profile for different durations of treatment up to 60 s.

For both devices conical tips were used with a comparable diameter (20 mm for thermal ablation, 19 mm for cryotherapy). We applied the probes on the tissue with a force of 10.8 N, measured by a weighing scale with an inaccuracy of 1 g and maximum weight of 5 kg. We performed the experiments in random order, with a minimum of 40 min between two cryotherapy measurements, and a minimum of 15 min between two thermal ablation measurements.

2.4 Data analysis

We recorded the minimum and maximum temperature for each thermocouple, and the change in temperature over time during and directly after the treatment with a National Instruments Temperature Input Module NI9211 in combination with Matlab R2017a. Every second two measurements were recorded. A moving average low-pass filter in Matlab with a window size equal to 10 and sample frequency of 2 Hz was used to smoothen out the data. The minimum and maximum temperature were defined as the minimum and maximum temperature measured during the application of the probe, after the moving average filter was applied.

The funder of this research had no role in the study design, data analysis or interpretation of results.

3 Results

A total of 7 experiments was conducted for each treatment modality. One experiment with cryotherapy was eliminated from analysis due to technical failure of the device during the experiment. Therefore, we analysed 6 experiments for cryotherapy and 7 experiments for thermal ablation.

3.1 Tissue-interactions cryotherapy

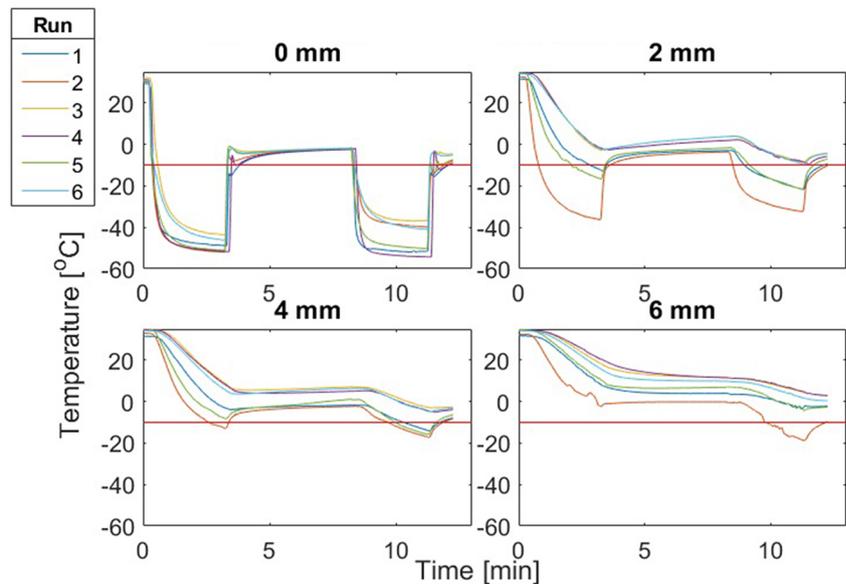
Figure 2 shows the temperature profiles of cryotherapy at the surface (0 mm) and a depth of 2, 4 and 6 mm. After a single freeze cycle the critical temperature to induce cell necrosis of -10 °C was not reached below the tissue surface. After the double freeze method the critical temperature was measured below the surface. Shortly after removing the probe, the temperatures increased.

Figure 3 displays the median minimum temperatures reached at the surface (0 mm) and at depths of 2, 4, and 6 mm. The critical temperature of -10 °C was reached in all experiments at the tissue surface, in 4 out of 6 experiments at 2 mm depth, in 3 out of 6 experiments at 4 mm depth, and 1 out of 6 experiments at 6 mm depth. The median temperature (median, IQR 25–75) at 0 mm depth was -51.9 °C (-51.9 °C to -46.3 °C), at 2 mm -15.7 °C (-21.7 °C to -9.8 °C), at 4 mm -9.6 °C (-15.8 °C to -4.9 °C), and at 6 mm -1.8 °C (-4.5 °C to 2.8 °C).

3.2 Tissue interactions thermal ablation

Figure 4 shows the temperature profiles of thermal ablation at the surface (0 mm) and a depth of 2, 4 and 6 mm. After 30 s of application, the critical temperature to induce cell necrosis of 46 °C was not measured below the tissue surface. After 45 s and 60 s the critical temperature was reached in the majority of

Fig. 2 Temperature profile of cryotherapy with double freeze method at depths of 0, 2, 4 and 6 mm. The red line indicates minus 10 °C, the critical temperature for tissue necrosis



experiments at 2 mm depth. The temperature continued to increase shortly after finishing the 60 s application. At 6 mm depth the critical temperature was never reached.

Figure 5 displays the median maximum temperatures reached at the surface (0 mm) and at depths of 2, 4, and 6 mm. The critical temperature to induce cell necrosis was reached in all experiments at the tissue surface, in 4 out of 7 experiments at a 2 mm depth, in 3 out of 7 at a 4 mm depth, and in none of the experiments at 6 mm depth. The median minimum temperature (median, IQR 25–75) at 0 mm depth was 87.9 °C (IQR 25–75 83.2 °C to 89.8 °C), at 2 mm 49.4 °C (IQR 25–75 43.2 °C to 55.8 °C), at 4 mm 43.1 °C (IQR 25–75 42.3 °C to 49.9 °C) and at 6 mm 37.5 °C (IQR 25–75 36.8 °C to 41.1 °C).

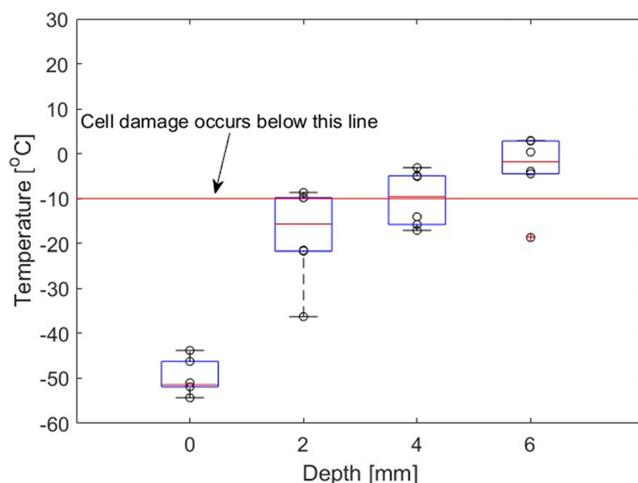


Fig. 3 Boxplot of minimum temperatures in 6 experiments with cryotherapy at depths of 0, 2, 4 and 6 mm. The red line in the box indicates the median minimum temperature

4 Discussion

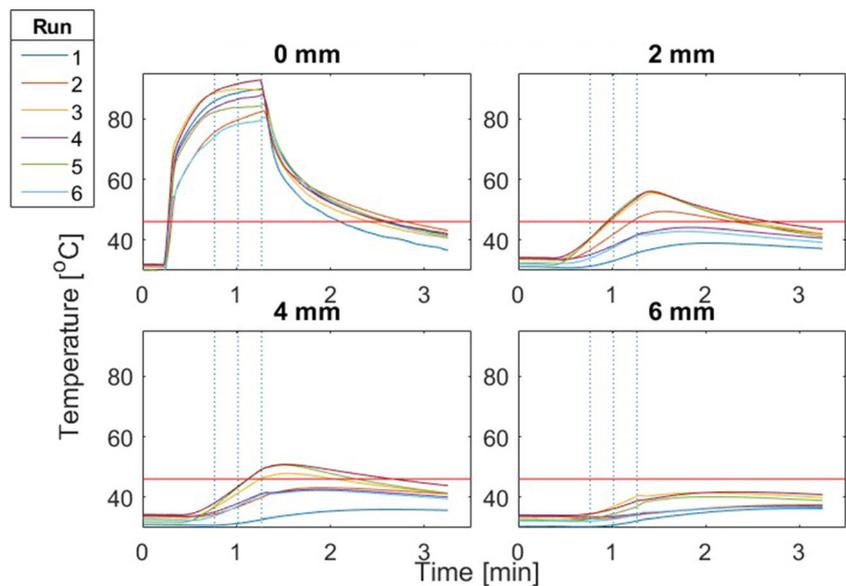
The aim of this study was to compare in an ex-vivo model the tissue interaction and temperature profile of both thermal ablation and cryotherapy. There is a lack of clinical studies comparing both treatment modalities and different treatment protocols for thermal ablation in comparable patient populations. To our knowledge this is the first ex-vivo study evaluating both treatment modalities in the same experimental setup. We hope our study will function as an inspiration towards the development of a standard treatment protocol for thermal ablation, which will support the implementation of thermal ablation and facilitate rolling out cervical cancer screening programs in LMICs.

4.1 Main findings

Temperatures to induce tissue necrosis were achieved at the tissue surface and in the majority of experiments at 2 mm depth for both cryotherapy and thermal ablation. For adequate treatment of CIN1, 2 and 3 lesions tissue necrosis at 4 mm is required [17, 18]. In our ex-vivo model at 4 mm depth the median minimum temperature of cryotherapy (−9.6 °C) and the median maximum temperature of thermal ablation (43.1 °C) were just below the critical temperatures for tissue necrosis (minus 10 °C and 46 °C respectively). After 30 s of thermal ablation the critical temperature for tissue necrosis was not achieved in any of the experiments.

Based on our findings we can conclude that both treatment modalities achieved tissue necrosis up to 4 mm in our ex-vivo model, although neither treatment modality achieved the median critical temperature at 4 mm depth. Application of thermal ablation for 30 s did not achieve adequate temperatures below the tissue surface and cryotherapy achieved tissue

Fig. 4 Temperature profile of thermal ablation with 100 °C for 60 s at depths of 0, 2, 4 and 6 mm. The red line indicates 46 °C, the critical temperature for tissue necrosis. The vertical lines indicate 30, 45, and 60 s duration of application of the probe on the tissue



necrosis at a deeper level than thermal ablation with single application.

4.2 Interpretation of results

Our findings are in line with the results of a clinical study conducted in Peru and El Salvador where patients planned for hysterectomy for benign indication were treated with cryotherapy or thermal ablation one day in advance [19]. This study evaluated the tissue necrosis depth 24 to 48 h after initial treatment. Cremer et al. reported a mean depth of tissue necrosis at 3.5 mm (range 0.9 mm) for thermal ablation (100 °C for 40 s), compared to 6.0 mm (range 1.6 mm) for double freeze cryotherapy, indicating that thermal ablation achieves less tissue necrosis depth than cryotherapy. In our model

thermal ablation was applied for a longer duration (60 s) compared to the study in Peru (40 s). However, even with application of 60 s, thermal ablation achieved slightly less tissue necrosis depth compared to cryotherapy.

Meta-analyses demonstrated that the double-freeze method of cryotherapy and thermal ablation are effective treatments for CIN lesions [10–13]. Our experiments underline the small differences in tissue depth necrosis between both treatment modalities. For cryotherapy more patient data are available compared to thermal ablation, and for thermal ablation data of different treatment protocols are pooled.

The application time of thermal ablation differs in clinical practice from 20s to 60s per treatment cycle. Based on Fig. 5 we can conclude that an application time of 45 s could induce cell necrosis at a tissue depth of 2 mm, but not at a tissue depth of 4 or 6 mm. Repeated cycles could achieve deeper tissue penetration.

A case series of Gordon et al. demonstrated a 92% cure rate after 5-years follow-up for CIN3 lesions treated with thermal ablation at 100 °C for 20 s with a total of 2 to 5 treatment cycles [20]. De Cristofaro et al. described that thermal ablation with 120 °C during 30 s achieved a tissue depth of 4 mm [21]. These findings combined with our study results suggest that a single application of 30 s at 100 °C seems to be insufficient to achieve adequate tissue depth necrosis, especially in high grade CIN lesions.

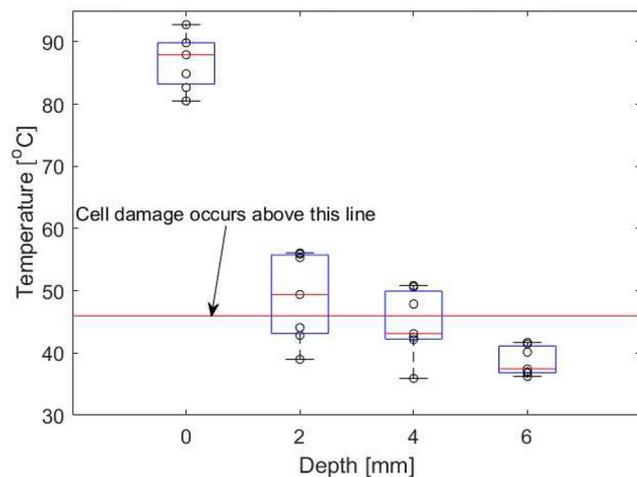


Fig. 5 Boxplot of maximum temperatures in 7 experiments with thermal ablation at depths of 0, 2, 4 and 6 mm. The red line in the box indicates the median maximum temperature

4.3 Limitations

Our findings in the ex-vivo model cannot be directly translated into clinical practice. Porcine tissue does not have the same thermal and biological properties as human cervical tissue and the ex-vivo model lacks vascularisation and perfusion, which influenced the temperature profile of the treatment methods.

We aimed to simulate the human body temperature by heating the tissue before application of the device, but it can only approximate the in-vivo situation. Therefore, the tissue depths where critical temperature for necrosis was reached, might differ from human cervical tissue. The depths of the needle thermocouples were an indication of tissue depth. The uncertainty of the insertion of the thermocouples was around 0.2 mm. A few times the probe shifted on the tissue for a maximum of 2–3 mm. We do not expect this has significantly influenced our measurements, since the surface of the whole probe is 19 or 20 mm.

The number of experiments in our study is small. Based on our findings when testing the ex-vivo model, conducting more experiments did not change the range in temperature profiles. Therefore, the number of experiments was not extended.

Despite these limitations, our study provides important insight in the tissue interactions and temperature profiles of thermal ablation and cryotherapy, by comparing both modalities under the same circumstances which cannot be measured in patients. We hope that this study can function as an inspiration or starting point for the WHO to develop a standard treatment protocol for thermal ablation, which will support the implementation of this treatment modality in LMICs, and will be a huge asset to the broad implementation of treatment programs for cervical cancer in LMICs.

4.4 Recommendations

For treatment of CIN 1, 2 and 3 lesions with cryotherapy the double freeze technique should be used. For treatment with thermal ablation a single application of less than 60 s might not be sufficient and multiple treatment cycles should be considered. We recommend to document findings including the treatment protocol from clinical studies evaluating different protocol in comparable patients and critically assess the persistence and recurrence of CIN lesions, especially for high-risk populations like women living with HIV infection.

4.5 Additional findings

In addition to the tissue interactions this study revealed differences in practicality and user friendliness of both treatment modalities.

4.5.1 Cryotherapy

The handles of the cryotherapy device to induce freezing and thawing are fragile and prone to breakage after frequent usage. During one of the treatment cycles, the handle of the cryotherapy device got frozen. As a result, the device could not be removed from the sample in the thawing period, and it kept freezing the tissue. This could cause discomfort to the patient

and will make it difficult for the health care provider to complete the double freeze method adequately. This problem has been reported by Maza et al. previously [7]. The pressure in the gas tank of cryotherapy significantly drops when used repeatedly on the same day, resulting in fluctuation of temperature of the cryotherapy probe. In our experiment we found that the temperature of the cryotherapy probe was not adequate after frequent use, although we used minimum intervals of 40 min in between the experiments and the pressure gauge of the gas tank indicated ‘normal pressure’. We used a small gas tank, in which the pressure can drop more easily while there is an adequate gas volume compared to a larger tank. Before every treatment the pressure in the gas tank needs to be checked and sufficient time in between treatments should be observed to avoid fluctuations in temperature.

4.5.2 Thermal ablation

The WISAP thermal coagulator has a timer indicating the duration of treatment. The sound of the timer has a low volume and could easily be unnoticed. An amplification of this beep or adding lights (1 light at 30 s, 2 lights at 45 s and 3 lights at 60 s) could help the health care provider to notice the alarm. We found that disinfection of the device is challenged by small irregularities in the design, causing dust and sand easily get stuck in the handle. Once the device is dysfunctional local repair is hardly possible and it needs to be replaced.

Availability of data and material The data from the experiments are available from the authors upon reasonable request.

Code availability Not applicable.

Authors' contributions Study conception and design was written by Marlieke de Fouw and Roos Marieke Oosting. Material preparation and data collection was performed by Dirk Eijkel and Pieter van Altena. Data analysis was performed by Marlieke de Fouw, Roos Marieke Oosting, Dirk Eijkel and Pieter van Altena. Jenny Dankelman, Alexander Arnold Willem Peters and Jogchum Jan Beltman reviewed the study design and data analysis. The first draft of the manuscript was written by Marlieke de Fouw and all authors commented on the previous versions of the manuscript. All authors read and approved the final manuscript.

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Compliance with ethical standards

Conflict of interest Author PA and DE have received honorarium from the Female Cancer Foundation for their work on this research. The other authors declare that they have no conflict of interest.

Ethics approval Not applicable.

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