Evaluation of a Novel EUS-Compatible Cryoablation Device for the In Situ Destruction of Pancreatic Cancer

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ABSTRACT

Cryoablation is an effective minimally invasive treatment for various cancers offering quicker recovery and reduced side effects. While promising, cryoablation of Pancreatic Cancer is limited by current technologies. In this study we evaluated a new, self-contained endoscopic ultrasound (EUS) compatible cryocatheter, *FrostBite*, for its potential to deliver targeted ablation via an EUS approach.

Thermal characteristics and ablative capacity were assessed using a heat loaded gel model, 3-dimensional tissue engineered models (TEMs), and a pilot porcine study. A single freeze protocol was evaluated.

Isotherm assessment revealed the generation of a 2.2cm dia frozen mass and the -20°C isotherm reaching 1.5cm following a 5 min freeze. TEM studies demonstrated attainment of ≤ -20°C at 1.9cm dia and fluorescent imaging 24 hr post-thaw revealed 2.0cm dia ablative zone (volume = 7.2 cm^3). Porcine studies demonstrated the consistent generation of 2cm x 3cm ablative areas.

Our results demonstrate the potential of EUS-based cryoablation and suggest that FrostBite may provide for rapid, effective, controllable freezing of pancreatic and liver tissue.

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INTRODUCTION

There is little question that new treatments are needed for pancreatic cancer (PaCa). Standard of care is surgical resection or chemotherapy followed by chemo-radiation therapy. Yet, in ~50% of patients with no metastases, tumor resection is not feasible due to vascular invasion, poor general health, or lacking surgical techniques. Cryoablation has been shown to be an effective treatment option for numerous cancers offering benefits such as shorter procedure times, cryolesion visualization, ability to target non-resectable tumors, reduced pain and operator stress, fewer unintended side-effects, among others.[1] The use of Cryo for PaCa has previously been reported[2-4], and while promising, remains investigational due to technological limitations.[5]

Through the use of a heated US gel model; 3-D tissue engineered model (TEM); and a pilot, porcine study, we evaluated *FrostBite* for its potential as an endoscopic compatible treatment option for the cryoablation of PaCa.

METHODS AND MATERIALS

FrostBite, an 8 Fr flexible cryocatheter with a 1.3mm (diameter) x 13cm (length) nitinol needle tip (3cm ablation segment), was connected to the PSN cryosurgical device. All studies were conducted using a single 3 or 5 min freeze cycle. Isotherm testing was conducted using a heat loaded US gel model and temperatures were recorded in real-time. TEM studies were conducted in a warm circulating bath with temperature recording. Iceball radii were measured at cardinal locations, then TEMs were placed into culture for viability assessment 1 day post-freeze using Calcein-AM and Propidium Iodide. TEMs were visualized using a Zeiss Axio Observer 7 and necrotic zones were determined using ZEN software. The pilot porcine study was performed at University Hospital's animal lab in the Cleveland Medical Center under IACUC approval. With an Olympus endoscopic ultrasound (EUS) gastroscope in place, *FrostBite* was inserted through the working channel. The EUS-scope was then maneuvered to visualize the liver and pancreas, then the needle tip was advanced under ultrasound. Freezing was applied and lesion formation was monitored in real time using US. Tissue was then excised with diameter and length of each lesion measured.

RESULTS

- Isotherm analysis revealed the generation of a 2.4cm diameter iceball with penetration of the -40 and -20°C isotherms to 1.5cm and 2.1cm, respectively.
- PaCA TEM studies revealed the creation of a 7.2cm^3 ablative volume (2cm diameter x 3.2cm long lesion).
- Porcine studies confirmed the consistent generation of a 2cm x 3.1cm ablative zone
- Destruction of tissue in close proximity to major vasculature was attained without damage to the vessel

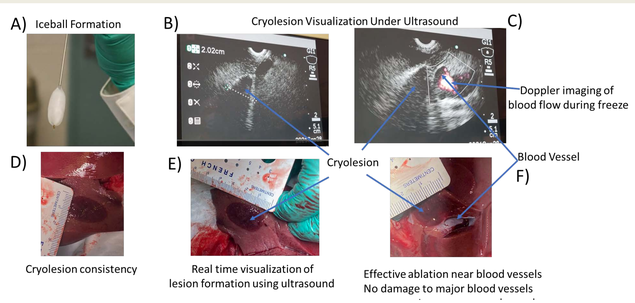
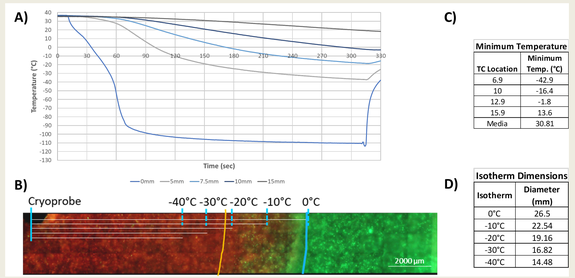


Figure 1. Representative Images of Porcine Liver Tissue Ablation.
A) Iceball created by *FrostBite* prior to EUS insertion. B) US image of iceball created during a 3 min freeze. C) Color Doppler US image showing proximity of iceball to major blood vessel demonstrating no obstruction of blood flow during the procedure. D) Gross pathology shows a deep red lesion post treatment. E) Representative image confirming creation of a 3cm long cryolesion. F) Cryolesion formation sculpting around a blood vessel.



C)

Minimum Temperature	
TC Location	Minimum Temp. (°C)
6.9	-42.9
10	-16.4
12.9	-1.8
15.9	13.6
Media	30.81

D)

Isotherm Dimensions	
Isotherm	Diameter (mm)
0°C	26.5
-10°C	22.54
-20°C	19.16
-30°C	16.82
-40°C	14.48

Figure 2. Assessment of Isothermal Profile and Ablative Zone Generated by *FrostBite*. A) Real-time temperature assessment in a heated US gel model revealed -20°C and -40°C isotherm penetration to an average diameter of 2cm and 1.5cm, respectively. B). Fluorescent image analysis of the PANC1 TEMs (24 hrs post-freeze) revealed a 1.8cm zone of complete cell death which correlated to ~-20°C (C,D). Staining Key: Green (CAM) = live cells; Red (PI)= dead cells; Blue arch= iceball edge; Orange arch= complete necrotic cell death edge.

DISCUSSION

While the use of percutaneous PaCa cryoablation has been reported[3,4], due to the anatomy, targeting PaCa using a percutaneous approach is challenging. Development of EUS-based cryotechnologies has been limited due to cryoprobe size and cryogen power limitations. Using a novel mixed phase nitrogen cryogen source (PSN), *FrostBite* was able to rapidly deliver sub-lethal temperatures through microtubing enabling the effective, repeatable ablation of tissue *in situ* via an EUS approach. Due to the small size of the pig pancreas, ablation studies were conducted on liver tissue. Analysis of the ablation efficacy revealed >70% destruction within the overall frozen mass compared to <40% reported for current percutaneous based cryodevices.

Table 1. Example Cryolesions Created by *FrostBite* in Porcine Liver Tissue

Lesion #	1	2	3	4	5	6	7	8
Freeze Protocol	Single	Single	Single	Single	Single	Single	Double	Single
Freeze Duration	3 min	3 min	3 min	3 min	3 min	3 min	3/3/3	3 min
Frozen Mass Size EUS - Diameter	N/A	2cm	2cm	1.8cm	1.99cm	N/A	1st Freeze: 2.1cm 2nd Freeze: 2.4cm	N/A
Length	N/A	N/A	3cm	N/A	3cm	N/A	2nd Freeze: 3.4cm	N/A
Pathology Diameter x Length	2.1cm x 3.2cm	2.1cm x 3.3cm	2cm x 3.1cm	2.2cm x 3.4cm	2cm x 3cm	N/A*	2.5cm x 3.5cm	N/A*

* Lesions 6 and 8 were found to overlap upon gross pathology analysis, thus measurement of lesion size was not recorded.

CONCLUSIONS

This study demonstrated *FrostBite* is able to rapidly and effectively freeze targeted tissue via an EUS approach. Cryolesion formation was successfully visualized in real time using ultrasound. Importantly, effective ablation was attained in close proximity to major vasculature without damaging the blood vessel. This may have implications for targeting non-resectable PaCa. Although further testing and refinement are needed, the results demonstrated the potential of *FrostBite* to provide a next-generation strategy for PaCa treatment.

REFERENCES

1. Baust JM, Rabin Y, Polascik T, Santucci KL, Snyder KK, Van Buskirk RG and Baust JG. Review Paper: Defeating cancers' adaptive defensive strategies using thermal ablative therapies. Technol. Cancer Res. Treat., 2018;17:1-16.
2. Baust JG, Gage AA, Robilotto AT, Baust JM. The pathophysiology of thermoablation: optimizing Tao Z, Tang Y, Li B, Yuan Z, Liu FH. Safety and effectiveness of cryosurgery on advanced pancreatic cancer: a systematic review. Pancreas. 2012 Jul;41(5):809-11
3. Chiu D, Niu L, Mu F, Peng X, Zhou L, Li H, *et al.* The experimental study for efficacy and safety of pancreatic cryosurgery. Cryobiology. 2010;60(3):281-6.
4. Kovach SJ, Hendrickson RJ, Cappadona CR, Schmidt CM, Groen K, Koniaris LG, *et al.* Cryoablation of unresectable pancreatic cancer. Surgery. 2002;131(4):463-4.
5. Baust JM, Robilotto, AT, Gage, AA, Baust JG. Enhanced Cryoablative Methodologies. In: Bischof JC, He X, eds. Multiscale Technologies for Cryomedicine: World Scientific Publishing Co; 2016. p. 388.