Peer Review File

Article Information: https://dx.doi.org/10.21037/atm-22-608

First Round of Peer Review

Reviewer# A:

Comment1：Recently, the risk of surgical smoke has attracted more and more attention. In this paper, it was suggested that the new plasma system produces less harmful by-products by analyzing surgical fumes. The experiment was well designed to collect smoke using a sealed container.

Generally, I think this paper is well prepared for publication.

Reply1: We thank the reviewer for reading our paper carefully and giving the above positive comments.

Comment2: In line 345, a fully closed system could avoid the escape of the smoke. This is very efficient way to analyze and measure the components. The conc of PM2.5 exceeded the limit of guideline.

However, in the actual operation room, vertical laminar flow and gas scavenger system may reduce the conc of particles or chemicals. Researches designed in consideration of these points can be regarded to be more practical. Please add some comments in discussion.

Reply2: We gratefully appreciate for your valuable suggestion. We totally agree with you that in the actual operation room, vertical laminar flow and gas scavenger system may reduce the conc of particles or chemicals. The reason we designed the surgical smoke analysis system in a fully closed container is that the key objective was to compare the surgical smoke generated during electrosurgery with hazardous compositions from the ES, the comparative PB and the NTS-100. However, your valuable suggestion is exactly our future research, as we plan to evaluate the smoke hazards of the electrosurgical devices in an actual operating room in future studies to assess the operator staff exposure. According to your suggestion, we have added some comments about it. (see Page 20, line 391-420)

Changes in the text: As shown in Figure 3, to conduct this experiment, we specially designed an entirely enclosed smoke generation, collection, measurement, and analysis system for the in-vitro experiment to ensure precision and accuracy. In addition to fully closed tissue dissection and smoke transportation procedure, the initial air is ejected from the container before the experiment by flushing helium. To our knowledge, the majority of surgical smoke collection devices used in previous studies could be broadly classified into two types based on the purpose of the researches: ① the researches aimed to evaluate the operator stuff exposure to surgical smoke would measure at levels approximating operator stuff's respiratory zone(11, 12). ② the researches aimed to evaluate the chemical compounds and particle matters generated in different cutting conditions (i.e. different tissues, different electrosurgical devices, and difference between endoscopic surgery and thoracotomy) would collect all generated

smoke as much as possible. Previous studies have used the grab sampling technique, which used evacuated canisters lined with fused silica. Also, the conductive tube and electrostatic precipitation collection device were used the force of an induced electrostatic charge to minimize particle electrical losses (13, 14). As with any surgical collection system stated in prior research, some particles may escape naturally away from these inlet areas and not be collected. (11-14). Considering the aim of this research was to compare the surgical smoke generated during electrosurgery with hazardous compositions from the ES, the comparative PB and the NTS-100, the surgical analysis system was designed to avoid the escape of the smoke. Similarly, in animal experiment, the gas collecting tube was tightly attached to the scalpel tip. In addition, this research analyzed particle size with the laser diffraction system (Microtrac S3500, range from 0.02-2800μm), avoiding underestimation of particle size, while most previously reported studies only detected particles with the upper range of 10μm (14). Apart from that, we conducted the experiments on anesthetized healthy swine, since the majority of reported studies were purely conducted on fresh tissues (13-15). Specifically, all incisions were made on the anesthetized swine to maximize the physiological mimicry of the actual surgical procedure.

Special thanks to you for your good comments.

Reviewer #B

Comment1: In the manuscript entitled "Surgical smoke analysis from a new surgical system that applies low-temperature plasma," the authors reveal that a NTS-100 system reduces hazardous smoke more than previous systems. I read your document with interest. There are a *few comments below.*

Reply1: We are very grateful to the reviewer for the time that you have spent in reviewing our manuscript and for the very wise comments.

Comment2：Is the NTS-100 system similar except that it is lower temperature than previous PB systems? Is there any difference in frequency etc. between the two systems?

Reply2：We sincerely appreciate your interest. The NTS-100 system showed lower local operating temperature than the previous PB systems. And the detailed difference in bleeding, histological scoring of injury, and wound strength were shown in Zhong Y, Wei Y, Min N, Guan Q, Zhao J, Zhu J, et al. Comparative healing of swine skin following incisions with different surgical devices. Ann Transl Med. 2021;9(20):1514. doi: 10.21037/atm-21-3090(1). We hope that this paper would meet your requirement. It would be our honor if you would like to spare your valuable time to read it.

Comment3：How is the hemostatic and coagulation power of the NTS-100 system compared to conventional electric scalpels and PB systems? Do you have data such as coagulation power of NTS-100 system in humans?

Reply3：We deeply appreciate your interest. The detailed information about the hemostatic and coagulation power of ES, PB, and NTS-100 could be found in Zhong Y, Wei Y, Min N, Guan Q, Zhao J, Zhu J, et al. Comparative healing of swine skin following incisions with different

surgical devices. Ann Transl Med. 2021;9(20):1514. doi: 10.21037/atm-21-3090 as well(1). This paper was attached.

Comment4：I think that it is very meaningful to show the data of VOC generation by electric scalpel. I also think that small particles cause respiratory illness and are extremely dangerous substances. This NTS-100 system contributes to the reduction of small particles. By the way, are large particles really less dangerous? The particle size is much larger than in previous PB systems, can this be explained solely by the temperature difference between the two systems?
Reply4: According to your suggestion, all the data of VOC would be presented in the text. (see table 6) As for the particle size, we hope the response below would be clear enough. The particles are classified as coarse (2.5–10μm), fine (0.1–2.5μm), and ultrafine (<0.1μm), where the degree of toxicity becomes greater for smaller particles. PM2.5 is dominated by products of combustion and secondary particles, while PM2.5–10 consists mainly of crustal, biological, and fine particle fraction components. Thus, smaller PM particles can penetrate deeply in the lungs, activating molecular mechanisms of epithelial and defense cells. The large particles (PM > 10μm) are generally filtered in the nose and throat and do not necessarily cause problems.(2) The harm of particulate matter are not fully clarified, but in terms of particle size, the available studies and reviews agree that the smaller the particulate matter the greater the health risk. We have added some comments. (see Page 22, line 452-463) Thank you for pointing out the reason for larger particles, which is exactly where we want to explore in the future. Based on the data, we only speculate that the tip temperature may be the reason for the problem, and hope that we could explain it in the future. According to your suggestion, we rewrote the sentences to avoid misunderstanding. (see Page 23, line 477-480)
Changes in the text:
The particles are classified as coarse (2.5–10μm), fine (0.1–2.5μm), and ultrafine (<0.1μm), where the degree of toxicity becomes greater for smaller particles (18, 19).
The large particles (PM > 10μm) are generally filtered in the nose and throat and do not necessarily cause problems (18).
Consequently, NTS-100 produced surgical smoke with a larger particle diameter. It is speculated that could be related to the fact that the NTS-100 working temperature (average tip temperature during cutting around 70°C) is lower than that of the PB (around 80°C). Further study is needed.

Once again, thank you very much for your comments and suggestions.

Reviewer #C
Comment1: The manuscript submitted has a lot of potential and contributes to further research on the topic.
Reply1: We appreciate your positive evaluation of our work. Thank you for your review.

Thanks for your encouraging comments.

Reviewer #D
Comment1: Overall - since there is no demonstration of harm or harm reduction in this

manuscript, I do not think study aims or conclusions about harm or potential hazard reduction can be made. The study compares particle mass and concentration, and volatile organic chemicals (VOCs), but does not demonstrate that the differences identified in these values lead to harm reduction.

Reply1: We would like to thank you for your careful reading and constructive suggestions. It is really as you suggested that there is no study on mutagenesis/carcinogenesis in this manuscript. We apologize for the unclear expression. According to your helpful advice, we have clarified the aim of this study was to compare the surgical smoke generated during electrosurgery with hazardous compositions from the ES, the comparative PB and the NTS-100. We have modified this expression throughout the text to clarify it. (see Page 5, line 32-35; Page 6, line 85-88) Existing studies on the risks of surgical smoke attribute the majority of the blame to PM, VOCs, and virus. This study didn't include any virus research. We add the discussion about this limitation and the comments on transmitted virus. (see Page 26, line 533-555) The harm of particle matters and VOCs have been demonstrated in a large number of previous studies in chemistry and medical fields. The permissible exposure levels recommended by OSHA in the US are available. The harm of the specific VOCs has been demonstrated in the screenshot attached below(3). However, the levels of exposure required to trigger mutagenesis/carcinogenesis have not yet been determined. We added some comments in limitation to be more clear. (see Page 28, line 565-568) Furthermore, because of the limitations of the measuring equipment, our study assessed VOCs qualitatively, which does not correspond to the recommended VOC level limits. We added some comments in discussion to be more clear. (see Page 28, line 565-568; Page 25, line 503-506) We apologize for the inconvenience and will work to enhance it in the future studies.

Table 1. Common Compounds Found in Surgical Smoke

Compound	Experimentally Recorded, ppm[a]	Recommended Exposure Limit, ppm[a]	Adverse Effects
Acetonitrile	190 (average)[25]	20 (average)[4]	Irritates nose and throat and can cause asphyxia; causes kidney and liver damage in animals[4,29]
Benzene	63.25 (maximum)[70]	0.008 (acute)[71]	Carcinogenic and mutagenic; increases risk of bone marrow failure and depression; trigger for leukemia; toxic to the central nervous system and can cause convulsions; can cause loss of consciousness and death[16,18,38]
Carbon monoxide	2200 (maximum)[33]	28.01 (acute)[71]	Can cause headache, vomiting, fatigue, and nausea; chronic exposure can cause heart problems (eg, cardiac dysrhythmia, myocardial ischemia, increased risk of heart disease); high levels can cause unconsciousness and death[29]
Ethylbenzene	0.031 (maximum)[41]	0.5 (acute)[72]	Causes respiratory effects (eg, throat irritation and chest constriction); causes neurologic effects (eg, dizziness and narcosis) and can lead to coma[4]
Formaldehyde	0.24 (maximum)[73]	0.05 (acute)[72]	Irritates eyes, nose, and respiratory system and can cause cough and bronchospasms; classified as a carcinogen and has been shown to cause nasal tumors in rats[29]
Hydrogen cyanide	25 (average)[74]	0.31 (acute)[71]	Easily absorbed by the lungs, gastrointestinal tract, and skin and inhibits cellular oxygen use; can cause asphyxia, headache, confusion, nausea, vomiting, and changes in respiratory rate[4]
Styrene	0.013 (average)[75]	5 (acute)[18]	Irritates eyes, nose, and respiratory system; possibly causes liver injury; adverse effects on nervous system include changes in vision, tiredness, concentration problems, slow reaction time, and balance problems[4]
Toluene	134 (maximum)[70]	1 (acute)	Can affect the nervous and cardiovascular systems; long-term occupational exposure to toluene can cause loss of vision, hearing, and muscle control, as well as brain damage and death; suspected to have further adverse effects on health when combined with alcohol or medications such as aspirin and acetaminophen[18]
Xylene	0.002 (maximum)[40]	1 (acute)[72]	Well absorbed by and can irritate the respiratory tract; chronic exposure is associated with reversible changes in red and white blood cell counts; can cause depression of the central nervous system that can lead to headache, irritability, depression, insomnia, tremors, impaired concentration and short-term memory, and reduced muscle control[29]

Abbreviation: ppm, parts per million.

[a] For parenthetical terms, maximum indicates the highest experimentally recorded value available; average indicates the value recorded when maximum values were not readily available; and acute indicates the recommended exposure limit measured at a single reading.

(3)

Changes in the text:

This research was designed to evaluate the surgical smoke generated during electrosurgery with hazardous compositions from the ES, the comparative PB and the new surgical system that

applies low-temperature plasma (NTS-100).

The study aimed to evaluate the surgical smoke generated during electrosurgery with hazardous compositions from the conventional high frequency electrotme, the comparative PlasmaBlade and the new surgical system that applies low-temperature plasma, using the in vitro and in vivo porcine model. We present the following article in accordance with the ARRIVE reporting checklist.

During the pandemic of COVID-19, Given the presence of HBV, HIV, and HPV in surgical smoke, it's reasonable to be concerned about the possibility of COVID-19 transmission (1). We presume that the low temperature of the tip would not promote the transmission of the virus. COVID-19 was detected in the peritoneal cavity, but the infective potential of surgical smoke remains unknown (22). To date, a study has found the presence of viral RNA of HCoV-299E (a human coronavirus representative) in surgical smoke with substantially decreased infectivity (22). Meanwhile, there has been a wide concern that cool aerosols are more likely to transport infectious and viable material than higher temperature aerosols (23, 24). This view needs to be considered suspiciously. Firstly, it was concluded from the research that the ES reduced the infectivity of surgical smoke more substantially than the ultrasonic scalpel, and speculated that the difference was related to lower surface temperature of the ultrasonic scalpel (23, 24). However, the difference of infectivity could also be explained by the low temperature vaporization of ultrasonic scalpel dissection. Instead of smoke, the ultrasonic scalpel generates vapor (23, 24). The unique atomization process may be responsible for the more biologically viable particles. Therefore, we speculate that the low temperature of the tip of the electrosurgical devices may not necessarily result in the transmission of the virus. A noteworthy outcome of this study was that surgical smoke generated by NTS-100 (2.5-122μm) was significantly larger in size than those produced by the US (0.35-0.75μm) and ES (0.07-042μm) (3). Further studies are needed to ascertain whether there is a risk of infectivity of NTS-100 during the pandemic of COVID-19.

Fourth, the VOCs were measured qualitatively and relatively quantitatively. Finally, further research on the mutagenesis or carcinogenesis caused by the ES, PB, and the NTS-100 in the operation theater to clarify its hazards to humans is needed.

Unfortunately, limited by detection instruments, only qualitative and relatively quantitative VOCs analysis were conducted in this research, which cannot be referenced to security standards or data from previous research.

Comment2: The authors should explain the value of measuring smoke in a closed environment or 5 cm away from the smoke source since these environments/circumstances are not encountered during surgery. The authors would benefit from revisions using a medical writer.

Reply2: Thanks for your comments. We apologize for any inconvenience caused by the unclear expressions in this manuscript. Comparison of the surgical smoke generated during electrosurgery with hazardous compositions from the ES, the comparative PB and the NTS-100 is the main objective of this paper. The reason we designed to measure smoke in a closed environment and the area closed to the scalpel tip was that we aimed to collect as much smoke as possible and detect all the components in the smoke. As for the 5cm, it was the closest distance that the PM2.5 sensor can be placed without disturbing the in vivo experimental operation. Also, to minimize the differences, the PM2.5 sensor was placed 5cm away from the

smoke source in the in vitro experiment. To be more clearly and in accordance with your concerns, more detailed discussion was added. (see Page 20, line 391-420)

Changes in the text:

To our knowledge, the majority of surgical smoke collection devices used in previous studies could be broadly classified into two types based on the purpose of the researches: ○1 the researches aimed to evaluate the operator stuff exposure to surgical smoke would measure at levels approximating operator stuff's respiratory zone (11, 12). ○2 the researches aimed to evaluate the chemical compounds and particle matters generated in different cutting conditions (i.e. different tissues, different electrosurgical devices, and difference between endoscopic surgery and thoracotomy) would collect all generated smoke as much as possible. Previous studies have used the grab sampling technique, which used evacuated canisters lined with fused silica. Also, the conductive tube and electrostatic precipitation collection device were used the force of an induced electrostatic charge to minimize particle electrical losses (13, 14). As with any surgical collection system stated in prior research, some particles may escape naturally away from these inlet areas and not be collected (11-14). Considering the aim of this research was to compare the surgical smoke generated during electrosurgery with hazardous compositions from the ES, the comparative PB and the NTS-100, the surgical analysis system was designed to avoid the escape of the smoke.

Comment3: 2.2.the air changes per hour in the room used to conduct the experiment, and the number of minutes permitted to elapse for clearing of surgical smoke between experiments should be reported. These factors should to be controlled for to make comparisons regarding air particles and VOCs.

Reply3 : Thanks for your comments. The original air in the closed container in the in vitro experiment was exhausted by the helium gas at a controlled flow rate of 5 ml/min for a total of 3 min between each experiment. In the animal experiment, continuous ventilation was present in the room, but not meeting the standards of the operating room, and the air changes per hour in the room was not recorded. We are sorry for this, but the purpose of this paper is to compare the surgical smoke generated by the ES, PB, and NTS-100. In addition, each step of the operation has a long gap. The baseline levels should be approximately the same. We have added some detailed methods hoping to make it clearer. (see Page 8, line 144-147)

Changes in the text: the gas collection outlet is used to connect the helium supply to the closed container, and the enclosed container is continuously filled with helium gas at a controlled flow rate of 5 ml/min for a total of 3 min until the original air inside the enclosed is exhausted.

Comment4: Figure 3, the description, and text should be revised to describe the experimental design such that a reader could repeat the experiment. It is unclear what materials were used, how smoke was collected into tubes, and how the smoke collected in tubes was tested without releasing smoke.

Reply4: Thanks for your suggestion. We are sorry that this part of information is not detailed enough. We have added detailed information (materials and procedure) in methods, hoping that it will help to repeat the experiment. (see Page 8-13, line 137-254) The text under Figure 3 have been revised according to your suggestion. (see Figure3) If the added information is not enough, we can consider including more literature on the methodology section and give more

details of our approach.

Changes in the text:

Figure3: Experimental settings for in vitro experiments. A, Surgical devices used in in vitro experiment; a, a new surgical system that applies low-temperature plasma; b, a comparative PlasmaBlade; c, a high frequency electrotome; B, Layout of surgical analysis system; C, Plan view of the experimental setting; a, electrosurgical equipment main unit; b, closed container; c, GC-MS(Gas Chromatography-Mass Spectrometry); d, TD(thermal desorption instrument); e, negative electrode plate wiring; f, electrosurgical equipment wiring; g, gas transportation wiring. The surgical analysis system operating process: Initially, the experimental tissues were placed in the closed container(b), the negative plate was tightly bonded to the tissues, and the lead from the negative plate and the electrosurgical was threaded through the adhesive plug and sealed. Then, the gas collection outlet(g) is used to connect the helium supply to the closed container, and the enclosed container is continuously filled with helium until the original air is exhausted. After that, incisions were made. Finally, an air collected pump was used to get all the newly produced smoke into the TD tubes(d), the closed air collecting bottle with ultrapure water or the empty air collecting bottle. In addition, the PM2.5 sensor was put into the sealed container for PM2.5 concentration measurement.

3. Surgical smoke analysis

3.1 in vitro experiment

Figure 3 demonstrated a fully closed surgical analysis system that we specially designed to collect as much surgical smoke as possible. Initially, the experimental tissues were placed in a closed container, the negative plate was tightly bonded to the tissues, and the lead from the negative plate was threaded through the adhesive plug and sealed. The electrosurgical surface is threaded through the other hole through the glue plug, and its length is tested (to the extent the surface can penetrate the tissue by 0.5 cm). Then, the gas collection outlet is used to connect the helium supply to the closed container, and the enclosed container is continuously filled with helium gas at a controlled flow rate of 5 ml/min for a total of 3 min until the original air inside the enclosed is exhausted. Then, we used the ES, PB, and NTS-100 to each make 10 incisions as mentioned above. After that, we used an air collected pump, get all the newly produced smoke into the TD tubes and empty air collecting bottles. After clearing up the remained smoke, we performed the same steps as before, collecting surgical smoke after 20 incisions were made. The collected bottle was filled with ultrapure water. After that, the PM2.5 sensor was put into the sealed container, and analyzed before and after cutting one incision using the ES, PB, and NTS-100.

3.1.1 VOCs measurement with PEN3: ① Assembling the system: the experimental tissues were placed in a closed container, the negative plate was tightly bonded to the tissues, and the lead from the negative plate was threaded through the adhesive plug and sealed. The electrosurgical surface is threaded through the other hole through the glue plug, and its length is tested (to the extent the surface can penetrate the tissue by 0.5 cm ; ② Evacuating existing gas: the helium gas supply is connected to the closed container using the gas collection port, and the container was continuously filled with helium gas at a controlled flow rate of 5 ml/min for 3 min until the existing air was exhausted; ③Tissue dissecting: the ES, PB, and NTS-100 were applied to the tissues at 1cm/s, and 10 parallel incisions of 1cm apart, 3cm long and 0.5cm deep were made; ④Gas collection: the surgical smoke was collected into a gas collection bottle

using a gas collection pump for 1 min; **5**Gas analysis: the gas collection bottle was connected to the sample tube, and the PEN3 airsensor (Beijing Ying Sheng Heng Tai Technology Co). was applied to detect the gas composition and save the images.

3.1.2 VOCs analysis with GC-MS: the procedure of assembling the analysis system, evacuating existing gas and tissue dissecting were the same as the PEN3 analysis as mentioned above. After the parameters of TD (7667A Thermal Desorber Agilent USA, Inc) are set, the surgical smoke was collected by the gas sample tube into the Thermal Desorber tube for sampling and concentrating lasted 5 min. At the end of sampling, the Thermal Desorber was in dry-blowing mode to remove the original ambient components; the Thermal Desorber was heated up and the surgical smoke was vaporized under high temperature and released into the GC-MS (5975T LTM-GC/MSD Agilent USA) for gas analysis.

3.1.3 Mean concentration of PM2.5: putting a PM2.5 sensor (LB-HD08, Lianyungang Arbor Electronics Technology Co) 5 cm from the tissue incisions in the sealed glassy container, the procedure of assembling the analysis system, and evacuating existing gas were the same as the PEN3 analysis as mentioned above. The ES, PB, and NTS-100 were applied to the tissues at 1cm/s, and 10 parallel incisions of 1cm apart, 3cm long and 0.5cm deep were made. And, we recorded the data by the PM2.5 sensor before cutting (p1) and 1min after cutting (p2). The concentration of the PM2.5 (p) =p2-p1.

3.1.4 Diameter distribution of particles: Examined the solution by the laser particle size analyzer (Microtrac S3500, US Magitek Co). The procedure of assembling the analysis system, and evacuating existing gas were the same as the PEN3 analysis as mentioned above. Next, the ES, PB, and NTS-100 were applied to the tissues at 1cm/s, and 20 parallel incisions of 1cm apart, 3cm long and 0.5cm deep were made. Then, the surgical smoke was collected for 10 min using an air pump into a gas collection bottle containing 40 ml of ultrapure water (ELGA purelab pulse, Elgar Ltd., UK). After that, diameter distribution of particles was measured by the laser particle size analyzer (Microtrac S3500, US Magitek Co), and each group of data was measured five times in total and the average value was taken.

3.1.5 Mass of particle measurement: Several clean microporous membrane filters (pore size 0.45μm Beijing Beihua Liming Membrane Separation Technology Co.) were taken, placed in a constant temperature and humidity cabinet, and weighed by parts per million microbalance (XP6, METTLER TOLEDO GmbH, Zurich, Switzerland) after 24h of equilibration under the equilibrium conditions (30°C, 45-55% humidity). Each filter membrane was weighed more than 10 times, and the highest and lowest values were excluded, and the average value of each filter membrane was the original mass of the membrane, which was recorded as m1. The procedure of the procedure of assembling the analysis system, evacuating existing gas and tissue dissecting were the same as the PEN3 analysis as mentioned above. After 10 min of sampling with the gas collection pump, the microporous membrane filters with filtered particles is taken off, equilibrated in a constant temperature and humidity chamber for 24 h, weighed more than 10 times, the highest and lowest values are excluded, and the average mass of each filter membrane recorded as m2, and repeated three times to take the average. The mass of particle (m)=m2-m1.

3.2Animal experiment

After the animals were anesthetized by a combination of ketamine hydrochloride and tamsulosin II (0.4 ml/kg) administered intramuscularly behind the neck, incisions were made

on the ventrimision of each animal, exposing target organs. The next step was to make incisions using the ES, PB, and NTS-100, successively. We then used an air collected pump to collect the newly produced smoke into the gas collected bottle. And all the experiment animals were euthanized after this research.

3.2.1 VOCs measurement with PEN3: ①The gas collection pump outlet is connected to the gas collection bottle by a rubber tube, and the suction port is connected to another rubber tube, and the rubber tube for collecting gas is tightly attached to the scalpel tip, in line with the scalpel tip moving velocity during the surgery. ② while operating, collect the surgical smoke, the scalpel finishes cutting, the gas collection is finished, pull out the rubber tube connected to the gas collection bottle, tighten the stopper of the gas collection bottle to ensure the airtightness of the gas collection bottle. ③Connect the inlet tube, connect the PEN3 airsense (Beijing Ying Sheng Heng Tai Technology Co) to the computer. ④ The computer screen displays the change of gas composition detected by each probe within 2 min, and saves the image.

3.2.2 Real-time concentration of PM2.5: putting a PM2.5 sensor (LB-HD08, Lianyungang Arbor Electronics Technology Co) at a distance of 5cm from cutting position with the same height level. The PM2.5 sensor measures the real-time PM2.5 concentration when cutting the tissue at the beginning of the surgery.

4.Analysis instrument

4.1 Thermal Desorber (TD) and Gas Chromatography Mass Spectra (GC-MS)

Gas analysis is performed in this experiment utilizing a GC-MS linked with a TD tube, and the GC-MS is divided into two parts, gas chromatography and mass spectrometry (8). A temperature-dependent variation in affinity exists between the mobile and stationary phases in gas chromatography, allowing for the sequential separation and analysis of a wide range of VOCs (8). GC-MS temperature rising procedure: 25°C keep 5min, rise to 80°C at 5°C/min, keep 0min, rise to 180°C at 20°C/min, keep 9min, total 30min (8). Under the operation of a magnetic or electric field, the GC-MS transforms molecules into ions, separating the mass-to-charge ratio (m/z) in time and space with the assistance of a high vacuum, and providing the molecular weight, formula for the compound, and structure (8). The TD tube (1/4 inch*7 inch, SEFM-G60200, Shanghai Amperex Scientific Instruments Co) is filled with the thin layer chromatography silica gel H (Type 60) 0.2355g (China Qingdao Ocean Chemical Group Corporation), which could extract the gas components into the sorbent packing, with sampling and concentration. The TD is then heated, and the gas extracted in it is volatilized by heat and released in the sorbent, which is then analyzed qualitatively and quantitatively in the GC-MS (8). TD heating up procedure: 25°C is kept for 3min, and it ramps up to 265°C at the rate of 200°C/min and keeps for 15.8min, running for 20min in total.

Comment5：3.2.2 - the authors should explain why measurements were taken from 5 cm from the surgical smoke source.

Reply5：Thanks for your carefully review. The 5cm was the closest distance that the PM2.5 sensor can be placed without disturbing the in vivo experimental operation. Also, to minimize the differences, the PM2.5 sensor was placed 5cm away from the smoke source in the in vitro experiment. We have added some methodological details. (see Page 10, line 179-186; see Page 12, line 231-234)

Changes in the text:

3.1.3 Mean concentration of PM2.5: putting a PM2.5 sensor (LB-HD08, Lianyungang Arbor Electronics Technology Co) 5 cm from the tissue incisions in the sealed glassy container, the procedure of assembling the analysis system, and evacuating existing gas were the same as the PEN3 analysis as mentioned above. The ES, PB, and NTS-100 were applied to the tissues at 1cm/s, and 10 parallel incisions of 1cm apart, 3cm long and 0.5cm deep were made. And, we recorded the data by the PM2.5 sensor before cutting (p1) and 1min after cutting (p2). The concentration of the PM2.5 (p) =p2-p1.

3.2.2 Real-time concentration of PM2.5: putting a PM2.5 sensor (LB-HD08, Lianyungang Arbor Electronics Technology Co) at a distance of 5cm from cutting position with the same height level. The PM2.5 sensor measures the real-time PM2.5 concentration when cutting the tissue at the beginning of the surgery.

Comment6：3.2.2 Were VOCs measured before and after experiments? If not, then results will not take to account VOCs already present in the environment.

Reply6: We deeply appreciate your careful review. We apologize for not measuring the VOCs already present in the environment. The aim of this study was to compare the surgical smoke generated during electrosurgery with hazardous compositions from the ES, the comparative PB and the NTS-100. The baseline levels of VOCs should be approximately the same because of the continuous ventilation system. We would improve this issue in future studies.

Comment7：1.1 the word "better" should be clarified - how is one result better than another? Background particle size and PM2.5 should be reported.

Reply7 : we agree with your assessment. Accordingly, throughout the manuscript, we have clarified the word "better". (see Page 15, line 290-294) Thank you for pointing this out. We agree that the background measurement is important. As the original air in the closed container in the in vitro experiment was exhausted by the helium gas at a controlled flow rate of 5 ml/min for a total of 3 min between each experiment. (see Page 9, line 160-163) Also, the aim of this study was to compare the surgical smoke generated during electrosurgery with hazardous compositions from the ES, the comparative PB and the NTS-100. The baseline levels should be approximately the same.

Changes in the text:

Specifically, the 122.223±34.034μm mean diameter of NTS-100 particles produced when dissecting skin with a minimum diameter greater than 10μm, is much larger than the 0.775±0.382μm and 2.507±0.277μm mean diameters of ES and PB; The mean diameter of NTS-100 particles dissected from muscle was 29.192±6.518μm, with a minimum diameter larger than 1μm, which was much larger than ES (0.942±0.298μm) and PB (2.625±0.639μm); the mean diameter of NTS-100 particles dissected from liver tissue was 2.585±0.784μm, smaller than PB (12.692±3.325μm) and larger than ES (0.988±0.690μm).

② Evacuating existing gas: the helium gas supply is connected to the closed container using the gas collection port, and the container was continuously filled with helium gas at a controlled flow rate of 5 ml/min for 3 min until the existing air was exhausted;

Comment8：1.2.2 - units of mass should be included. the kind of filters and the method of injection should be reported in the methods. Was tissue weighed before and after experiments

to compare the amount of desiccated tissue with each experiment?

Reply8: Thank you for pointing this out. We have added the unit of mass throughout the manuscript. And unit of concentration have been added either. (see Page 17, line 331-342; see Page 16-17, line 304-329) The tissue was not weighed before and after experiments. It would be helpful to compare the amount of desiccated tissue. However, in our study, the mass of the particles was relatively low compared to the weight of the tissue, so we have not conducted the experiment. And we could explore it in the future study according to your suggestion.

Changes in the text:

1.3 Mass of particle measurement

By weighing filters (Sup 2), we measured the mass of the particles. After fully stoving, the smoke is injected through a particle filtering membrane and weighed. Figure 5 and Table4 depicted the comparison of ES, NTS-100, and NTS-100. There is a significant difference between the mass of surgical smoke generated by NTS-100 in liver tissues (0.093±0.004mg), skin tissues (0.160±0.004mg) and muscle tissues (0.176±0.038mg). There was no statistically significant difference in the mass of particles produced when ES was used to cut three different tissues. In addition, the NTS-100 generated significantly fewer particles than ES (liver: 0.263±0.053mg, skin: 0.304±0.007mg, muscle: 0.337±0.048mg, respectively) ($P<0.05$), but no differences in particle mass were observed between PB and NTS-100 (liver: 0.089±0.006mg, skin: 0.146±0.010mg, muscle: 0.181±0.059mg, respectively) ($P>0.05$).

1.2 concentration of PM2.5

1.2.1 animal experiment

The average PM2.5 concentrations produced by nine different cutting settings are shown in Table 2. The smoke observed during operation procedure is shown in Sup 1. The real-time PM2.5 concentrations of animal experiment were detected using the PM2.5 sensor at the same height and distance from cutting position. The average PM2.5 concentration generated by NTS-100 cutting muscles (1483.67±40.75μg/m^3) was significantly higher than that generated by cutting skin tissues (1079.00±81.68μg/m^3) ($P<0.05$). Similarly, the PM2.5 concentration produced by NTS-100 cutting skin tissues was significantly higher than the concentration produced by cutting liver with NTS-100 (924.67±79.22μg/m^3). Likewise, PB yielded the same results. ES, on the other hand, did not distinguish between three tissues ($P>0.05$). Furthermore, the concentrations of PM2.5 produced by NTS-100 were significantly lower than that of the ES (liver: 3547.50±45.40μg/m^3, skin: 3913.50±40.86μg/m^3, muscle: 3742.25±42.25μg/m^3, respectively) ($P<0.05$), but not significantly different from that produced by the PB (liver: 913.00±87.41μg/m^3, skin: 1018.00±29.16μg/m^3, muscle: 1534.33±61.06μg/m^3, respectively) ($P>0.05$).

1.2.2 in vitro

PM2.5 concentrations were measured with PM2.5 sensors placed in the closed container. It was observed that the PM2.5 concentration generated by the NTS-100 cutting muscle (2757.33±62.90μg/m^3) was approximately two and a half times greater than that generated by the liver (1148.33±63.61μg/m^3) ($P<0.01$), whereas no statistically significant difference was observed between the skin (2298.33±41.79μg/m^3) and liver or muscle tissues ($P>0.05$). By contrast, there was no significant difference in the PM2.5 concentration generated by the ES cutting three tissues ($P>0.05$). It should be noted that the concentrations of PM2.5 produced by NTS-100 were significantly lower than those produced by the ES (liver: 4124.50±91.25μg/m^3,

skin: 4200.25±68.72μg/m³, muscle: 4156.60±21.40μg/m³) (P<0.05) and were comparable to those produced by the PB (liver: 1192.00±53.37μg/m³, skin: 2203.67±97.19μg/m³, muscle: 2646.33±45.11μg/m³) (P>0.05).

Comment9 2.1.1 - units of measure should be included with the VOCs. Background VOCs prior to experiments should be reported.,

Reply9: We deeply appreciate your suggestion, and apologize for our negligence. The unit of VOCs measured by GC-MS has been added, and the VOCs measured by PEN3 is a scale value. (see Page 19, line 380-383) The aim of this study was to compare the surgical smoke generated during electrosurgery with hazardous compositions from the ES, the comparative PB and the NTS-100. In the animal experiment, the baseline levels of VOCs should be approximately the same because of the continuous ventilation system. We would improve this issue in future studies.

Changes in the text:

2.2 GC-MS of in vitro experiment

Specific VOCs of in vitro experiment were evaluated with GC-MS. Table6 showed smoke composition and volume concentration produced in nine cutting conditions. All the cutting modes on muscles produced more types of chemical components than on the skin and liver. In particular, NTS-100 generated hydrocarbons and aldehydes while skin did not, and alcohols, sulfides, and aldehydes while liver did not. In comparison to PB and ES, NTS-100 generated less harmful components and had a lower concentration of surgical smoke than ES (P<0.05), with no discernible differences from PB (P>0.05). In particular, for the detection of aromatic compounds, NTS-100 yielded much lower concentrations of VOCs (liver: 8.74±1.36%, skin: 7.15±1.83%, muscle: 5.04±0.88%, respectively) than ES (liver: 21.12±2.38%, skin: 18.07±4.47%, muscle: 15.66±2.42%, respectively) (P<0.05), and below PB (liver: 10.36±2.41%, skin: 9.54±2.66%, muscle: 10.03±1.34%, respectively) (P>0.05) in all experiments.

Comment10：discussion：The authors did not compare in vivo and in vitro particle creation.

Reply10: We are sorry that we failed to make us clear. Hope the revision could meet the requirements. We have added these contents in the discussion sector of this revision. (see Page 25, line 507-516) However, it is important to emphasize that the particle matters between in vivo and in vitro could not been compared directly, as the experimental conditions and methods were very different. So we compared the performance of the ES, PB, and NTS-100 in in vivo and in vitro experiment respectively. and then we found that the NTS-100 produced fewer and lower concentrations of VOCs than the PB and ES in animal experiment, while no significant difference was observed between the NTS-100 and the PB in the in vitro experiment. As for the PM2.5 concentration measured in the animal experiment, no significant difference was observed between the NTS-100 and the PB, just like in the in vivo experiments. We speculate that might result from the in-vivo blood supply affecting the types and concentrations of VOCs. Additional studies are required.

Changes in the text:

Interestingly, in the animal experiments, the NTS-100 generated less types and lower concentrations of VOCs than PB, while that of VOCs were similar in the in vitro experiment.

In the animal experiment, the NTS-100 only generated ammonia oxides and methane when cutting different tissues. That might result from the in-vivo blood supply affecting the generation of particulate aerosols. Consequently, it is assumed that NTS-100 may perform effectively in the clinic. However, as for the PM2.5 concentration measured in the animal experiment, no significant difference was observed between the NTS-100 and the PB, just like the results of the in vivo experiments. Also, escaping smoke in animal experiments undermined the plausibility of the conclusion. Additional studies are required.

Comment 11: Discussion regarding superiority of one method over another is not supported by the data. The authors suggest that their data support that the particles created during these experiments cause mutagenesis and carcinogenesis, but it should be noted that no experiments showing mutagenesis or carcinogenesis were conducted. There should be a discussion about how levels they measured compare to the levels/duration of exposure needed to cause mutagenesis/carcinogenesis.

Reply11: Thank you for pointing out the potential for misunderstanding. The conclusion has been clarified that the NTS-100 generated less hazardous components in surgical smoke than the conventional ES and performed comparably with the comparative PB. The results showed that compared to the ES the surgical smoke produced by the NTS-100 had fewer types and lower concentrations of harmful VOCs, larger particles, lower PM2.5 concentration, and lower particles mass. We have considered it sufficient to prove that the NTS-100 generated less hazardous compositions than the ES. In addition, the NTS-100 generated larger particles than the ES and PB. And there is no significant difference between the PB and the NTS-100 in terms of VOCs, PM2.5 concentration, and mass of particles. In general, the NTS-100 was comparable to the PB. As you suggested, we have clarified the aim of this manuscript: Comparison of the surgical smoke generated during electrosurgery with hazardous compositions from the ES, the comparative PB and the NTS-100. The conclusion has been clarified that the NTS-100 generated less hazardous components in surgical smoke than the conventional ES and performed comparably with the comparative PB. We have modified this expression throughout the manuscript. (see Page 6, line 85-88; see Page 28, line 575-578; see Page 28, line 582-585) We agree that no experiments showing mutagenesis or carcinogenesis were conducted. The mutagenesis or carcinogenesis of particle matters and VOCs (acetonitrile (chloromethane), benzene, co, ethylbenzene, formaldehyde, styrene, toluene, and xylene) have been reported in previous studies yet. We hope the screenshot attached below would be clear enough(3). Research on the carcinogenicity of smoke from the ES, PB, and NTS-100 could be conducted in further studies. Also, only qualitative and relatively quantitative VOCs analysis were conducted in this research, which cannot be referenced to security standards or data from previous research. According to your suggestion. We added to discussion hoping to make it clearer. (see Page25, line 503-506; see Page 28, line 565-568)

Table 1. Common Compounds Found in Surgical Smoke

Compound	Experimentally Recorded, ppm[a]	Recommended Exposure Limit, ppm[a]	Adverse Effects
Acetonitrile	190 (average)[25]	20 (average)[4]	Irritates nose and throat and can cause asphyxia; causes kidney and liver damage in animals[4,29]
Benzene	63.25 (maximum)[70]	0.008 (acute)[71]	Carcinogenic and mutagenic; increases risk of bone marrow failure and depression; trigger for leukemia; toxic to the central nervous system and can cause convulsions; can cause loss of consciousness and death[16,18,38]
Carbon monoxide	2200 (maximum)[33]	28.01 (acute)[71]	Can cause headache, vomiting, fatigue, and nausea; chronic exposure can cause heart problems (eg, cardiac dysrhythmia, myocardial ischemia, increased risk of heart disease); high levels can cause unconsciousness and death[29]
Ethylbenzene	0.031 (maximum)[41]	0.5 (acute)[72]	Causes respiratory effects (eg, throat irritation and chest constriction); causes neurologic effects (eg, dizziness and narcosis) and can lead to coma[4]
Formaldehyde	0.24 (maximum)[73]	0.05 (acute)[72]	Irritates eyes, nose, and respiratory system and can cause cough and bronchospasms; classified as a carcinogen and has been shown to cause nasal tumors in rats[29]
Hydrogen cyanide	25 (average)[74]	0.31 (acute)[71]	Easily absorbed by the lungs, gastrointestinal tract, and skin and inhibits cellular oxygen use; can cause asphyxia, headache, confusion, nausea, vomiting, and changes in respiratory rate[4]
Styrene	0.013 (average)[75]	5 (acute)[18]	Irritates eyes, nose, and respiratory system; possibly causes liver injury; adverse effects on nervous system include changes in vision, tiredness, concentration problems, slow reaction time, and balance problems[4]
Toluene	134 (maximum)[70]	1 (acute)	Can affect the nervous and cardiovascular systems; long-term occupational exposure to toluene can cause loss of vision, hearing, and muscle control, as well as brain damage and death; suspected to have further adverse effects on health when combined with alcohol or medications such as aspirin and acetaminophen[18]
Xylene	0.002 (maximum)[40]	1 (acute)[72]	Well absorbed by and can irritate the respiratory tract; chronic exposure is associated with reversible changes in red and white blood cell counts; can cause depression of the central nervous system that can lead to headache, irritability, depression, insomnia, tremors, impaired concentration and short-term memory, and reduced muscle control[29]

Abbreviation: ppm, parts per million.

[a] For parenthetical terms, maximum indicates the highest experimentally recorded value available; average indicates the value recorded when maximum values were not readily available; and acute indicates the recommended exposure limit measured at a single reading.

(3)

Changes in the text:

The study aimed to evaluate the surgical smoke generated during electrosurgery with hazardous compositions from the conventional high frequency electrotme, the comparative PlasmaBlade and the new surgical system that applies low-temperature plasma, using the in vitro and in vivo porcine model.

This research demonstrated that NTS-100 generated less hazardous by-products of surgical smoke substantially. Our team believes that the NTS-100 has a promising future. Then, further clinical data will be compared to current electrosurgical devices in order to continuously improve our system.

Conclusion:

In this research, hazardous chemical compounds and particle matters were detected in surgical smoke. It is suggested that the NTS-100 generated less hazardous components in surgical smoke than the conventional ES and performed comparably with the comparative PB. It is speculated that the application of NTS-100 may reduce the potential hazards to operating room personnel of surgical smoke.

Most of the compounds detected in this research are classified as hazardous to human health, and levels found in previous studies exceed recommended limits (3). In addition, the most commonly reported components of surgical smoke include acetonitrile (chloromethane), benzene, co, ethylbenzene, formaldehyde, styrene, toluene, and xylene, which are mostly aromatic compounds. Particular attention should be paid to aromatic compounds, known carcinogens. As a result of acute intoxication with aromatic compounds, one may experience vertigo and headaches, tachycardia, arrhythmia, trembling, disorientation, impaired consciousness, or even death. Also, benzene accounts for the largest proportion of aromatic compounds (15), and exposure to benzene is associated with incident myelodysplastic syndromes and T-cell lymphoma and cause damage to the central nervous system as well as

loss of consciousness (3, 19). In this study, the proportion of aromatic compounds also ranked high among all chemical compounds, and benzene was detected by GC-MS. Notably, NTS-100 generated less aromatic chemical compounds than ES and PB in all experiments. We speculate that the lower tip temperature of NTS-100 may explain it. Unfortunately, limited by detection instruments, only qualitative and relatively quantitative VOCs analysis were conducted in this research, which cannot be referenced to security standards or data from previous research.

Fourth, the VOCs were measured qualitatively and relatively quantitatively. Finally, further research on the mutagenesis or carcinogenesis caused by the ES, PB, and the NTS-100 in the operation theater to clarify its hazards to humans is needed.

Comment12：discussion：The authors suggest that the quantities of VOCs produced during their experiments cause a number of human ailments. There should be a discussion about how levels they measured compare to the levels/duration of exposure needed to cause these human ailments.

Reply12: It is really true as you suggested that a discussion about how levels measured compare to the levels/duration of exposure is needed. Sadly, the VOCs was measured qualitatively and relatively quantitatively with unit of G/G_0 and %. As the lack of accurate quantified VOCs, the results cannot reference to security standards or data from previous research (unit of VOCs: ppm). Thanks for your reminding, we added to the discussion. (see Page25, line 503-506)

Changes in the text: Unfortunately, limited by detection instruments, only qualitative and relatively quantitative VOCs analysis were conducted in this research, which cannot be referenced to security standards or data from previous research.

Comment13：discussion：The data described in this manuscript do not measure ability to aerosolize virus, therefore conclusions about the ability of one device to aerosolize virus over another should not be made.

Reply13: Thanks for your suggestion. We apologize for the misunderstanding induced by our phrasing. We have revised this part (see Page26, line 533-555) Actually, the aim of this part is to illustrate the lower tip temperature of PlasmaBlade is not necessarily associated with the easier virus transmission. The reason I discuss the ability to aerosolize virus is as follows: during the COVID-19 pandemic, the risk of virus transmission is an important topic. And the average temperature tip of the NTS-100 is 70 ℃， Meanwhile, there has been a wide concern that cool aerosols are more likely to transport infectious and viable material than higher temperature aerosols. This part is also just a speculation with the intention of not associating temperature with transmitted viruses, so the NTS-100 should not be blamed for the infectivity. If this part is still inappropriate, I could eliminate it. Research on transmitted viruses could be done in future studies according to your suggestion.

Changes in the text:

During the pandemic of COVID-19, Given the presence of HBV, HIV, and HPV in surgical smoke, it's reasonable to be concerned about the possibility of COVID-19 transmission (1). We presume that the low temperature of the tip would not promote the transmission of the virus. COVID-19 was detected in the peritoneal cavity, but the infective potential of surgical smoke

remains unknown (22). To date, a study has found the presence of viral RNA of HCoV-299E (a human coronavirus representative) in surgical smoke with substantially decreased infectivity (22). Meanwhile, there has been a wide concern that cool aerosols are more likely to transport infectious and viable material than higher temperature aerosols (23, 24). This view needs to be considered suspiciously. Firstly, it was concluded from the research that the ES reduced the infectivity of surgical smoke more substantially than the ultrasonic scalpel, and speculated that the difference was related to lower surface temperature of the ultrasonic scalpel (23, 24). However, the difference of infectivity could also be explained by the low temperature vaporization of ultrasonic scalpel dissection. Instead of smoke, the ultrasonic scalpel generates vapor (23, 24). The unique atomization process may be responsible for the more biologically viable particles. Therefore, we speculate that the low temperature of the tip of the electrosurgical devices may not necessarily result in the transmission of the virus. A noteworthy outcome of this study was that surgical smoke generated by NTS-100 (2.5-122μm) was significantly larger in size than those produced by the US (0.35-0.75μm) and ES (0.07-042μm) (3). Further studies are needed to ascertain whether there is a risk of infectivity of NTS-100 during the pandemic of COVID-19.

Second Round of Peer Review

Comment1：Photos of the full experimental set up with labels (instead of the cartoon schematic in figure 3) would greatly facilitate understanding of the methods used for collecting and measuring surgical smoke components.
Reply1: Thanks for your comments. We' re sorry that no photos of the full experimental set up could be provided.

Comment2: Particle size did not appear to be normally distributed in figure 4. Therefore, mean size may not be the most appropriate metric for reporting or comparison; median with range may be more appropriate. Consultation with a statistician should be considered.
Reply2: Thanks for your comments. The aim of this study was to compare the particle matters of three electrosurgical devices. The particle sizes of three three electrosurgical devices were analyzed with the same metric. As the figure 4 shows, the PB and NTS-100 produced larger particles than the ES. And the particles generated by the NTS-100 when cutting skin were apparently larger than the PB and the ES.

Comment3: For the animal experiments, stating that ventilation in the room was not measured, and that baseline levels were not measured before each experiment is important.
Both factors need to be noted as significant limitations to the interpretation of this study. The authors note in their response that there was a long gap between each step of operations, but this does indicate knowing if baseline PM or VOCs varied between experiments, since the time between steps was not standardized, the amount of PM or VOCs after each experiment was not known (which may vary based on previous experiments, different personnel, or different pigs), and the ventilation of the room was not known or standardized.

Reply3: Thanks for your careful review and valuable suggestion. It has been stated in the limitations section that the room ventilation and baseline levels were not measured before each experiment. We have modified our text as advised. (see Page25, line 511-513)
Changes in the text: First, in animal experiments, the ventilation of the room and the baseline level before each experiment were not measured.

Comment4: For the discussion, the authors should note that nearly all substances can be harmful to humans in sufficient quantities, and that harm is related to level of exposure. Stating that the quantity of particles measured in a closed system exceeded air quality guidelines (line 426) is misleading because the particles in the closed system do not represent what humans are exposed to while using electrocautery in the operating room. Extensive discussion of how PM and VOC affect human health (lines 462-473, 491-500) should be avoided because data related to this were not presented in the manuscript and the quantities measured in this manuscript's experiments are not reflective of human exposure, and therefore, again, could be misleading.
Reply4: Thanks for your comments. According to your suggestion, the extensive discussion of how PM and VOCs affect human health has been removed to avoid misleading. We have modified our text as advised. (see Page21, line 420-421; Page23, line 455-460; Page24, line 476-477)
Changes in the text:
The average diameters of particles were all below 1μm.
In addition, particles with a diameter of less than 2.5μm can absorb VOCs such as polycyclic aromatic hydrocarbons, benzene, as well as heavy metals (3, 20). The average diameter of NTS-100 cutting liver tissues was 2.585±0.784μm, significantly larger than the particle generated by the ES(0.988±0.690μm). Furthermore, ultrafine particles generally defined as particles with a diameter less than 0.1μm, are capable of penetrating cells (20, 21).
Most of the compounds detected in this research are classified as hazardous components to human health when exceeding recommended limits (3).

Comment5: Since no assessment of viral aresolization was made in this manuscript, the authors should consider removing mention and speculation related to COVID-19 and other viruses and their potential for areosolization during surgery (lines 534-556) since it is not relevant to the data presented in this manuscript. The one exception would be to mention that assessment of viral aresolization was not performed and therefore is a limitation of the study's ability to evaluate potential hazards from surgical smoke.
Reply5: We gratefully appreciate for your valuable suggestion. We have removed the discussion about covid19 and added some notes about covid-19 to the limitation. We have modified our text as advised. (see Page25, line 518-519)
Changes in the text: Finally, further studies are needed to ascertain whether there is a risk of COVID-19 when operating with NTS-100.

Comment6: Line 88 - "the" should be changed to "an"
Reply6: Thank you for pointing this out. We have corrected it. (see Page6, line 83)
Changes in the text: using an in vitro and in vivo porcine model

Comment7: Line 116-117 - I am not familiar with cut mode 6 and cut 45 mode. Can the meaning of these numbers be further defined? What is their unit of measure, and where do these numbers fall relative to typical numbers used in surgery?

Reply7: Thanks for your careful review. The cut mode 6 and cut mode 45 are the most typical modes used in the operation. The parameters of them are complicated. We have modified our text. (see Page 7, line 114).

Changes in the text: These are the most typical modes used in the operation.