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Oncological Safety of Ultrasonically Activated Surgical Devices During Gastric Cancer Surgery

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Abstract. *Background/Aim: Ultrasonically activated surgical devices (USADs) have become indispensable instruments for gastrointestinal surgery. In this study, we investigated the oncological safety of the use of USADs. Materials and Methods: We harvested and cultivated the splashes and mist scattered from an USAD when cutting MKN45-derived cancer nodules. Seven days later, we observed viable cancer cells and the total number of cells was counted. The histopathology of the nodules cut by the USAD was also examined. Results: The existence of viable cancer cells was confirmed by ex vivo cell culture. The number of viable cancer cells was reduced by slow grasping of the USAD. The surface of cancerous tissue cut by the USAD was partially heat-denatured, however, there were some parts in which cancerous tissue was exposed on the surface. Conclusion: Surgeons should recognize the possibility that cancer cells may be scattered by USAD use.*

In recent years, the progress made in the development of energy devices for laparoscopic surgery has been remarkable. Ultrasonically activated surgical devices (USADs) have become indispensable for both laparoscopic and open gastrointestinal surgery. USADs exert two major functions. One is protein denaturation by frictional heat, which forms a sticky coagulum that coats blood and lymphatic vessel walls. The other is mechanical vibration, which cuts tissue by expansion and contraction (1, 2). Few papers regarding the oncological safety of USADs have been reported. Previously, several reports on port-site recurrence after laparoscopic surgery suggested intra-operative iatrogenic cancer spread, but the mechanism is unclear. These reports implicated that USADs could cause iatrogenic cancer to spread because one of the big differences between laparoscopic and open surgery is the frequent use of USADs in the former. Other mechanisms were thought to cause peritoneal recurrence, such as carbon dioxide pneumoperitoneum during the laparoscopic phase (3, 4), tumor perforation, and inadequate manipulation by hard forceps or contamination of instruments (5). However, with improvements in laparoscopic skills amongst surgeons and the increasing use of USADs in open surgery, several recent papers suggested that recurrence or metastasis will rarely occur following the application of laparoscopic approaches (6, 7). Most surgeons are under the impression that cancer cells will be killed by the heat of USADs even though they activate and cut cancerous tissues. However, we found that the mist generated by USADs, which includes splashes resulting from ultrasonic vibration, began to diffuse just after activation, and speculate that the cancer cells in this mist might be viable, because this process occurs before heat elevation. If this hypothesis is real, surgical oncologists may possibly disseminate viable cancer cells in the peritoneal cavity when using these beneficial and convenient devices.

Thus, the aim of this study was to investigate the oncologically safe use of USADs in gastric cancer surgery.

Materials and Methods

USAD. The USAD used in our experiments was the Harmonic Ace+ (Ethicon ENDO-Surgery, Cincinnati, OH, USA).

Mice. Eight- to 10-week-old female nude mice (Shimizu Laboratory Supplies, Kyoto, Japan) were used as the peritoneal dissemination model in this study. Mice were housed under specific pathogen-free conditions.

Cell line. The human gastric cancer cell line; MKN45 was cultured in RPMI-1640 (Nacalai Tesque, Kyoto, Japan) supplemented with

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100 IU/ml penicillin, 100 μg/ml streptomycin (Nacalai Tesque), and 10% heat-inactivated fetal bovine serum (Gibco, Thermo Fisher Scientific, Grand Island, NY, USA). The cells were maintained at 37˚C in 5% CO_2 and passaged every 3 days.

Cancer cell scattering test. We developed peritoneal dissemination models by performing intra-abdominal injection of 1.0×10^6 MKN45 cancer cells [300 μl in phosphate buffered saline (Nacalai Tesque)] in nude mice. Four weeks later, the mice were killed and disseminated tumor nodules were removed. Nodules were cut in a 50-ml tube using USAD, and the splashes and mist attached on the surface of the tube were rinsed, harvested, and cultivated in culture media at 37˚C in 5% CO_2 atmosphere as shown in Figure 1. At that time, we set two conditions: In the slow grasp group, to avoid crushing the tumor, we grasped the tumor slowly and activated the USAD at the same time; In the destructive grasp group, we grasped and crushed the tumor, and then activated the USAD. As a control tissue, murine non-cancerous omentum was used. Seven days later, we observed *ex vivo* viable cancer cells by light microscopy. After 3-4 days of cultivation, all cells were harvested using 2.5g/l trypsin/1 mmol/l EDTA solution with phenol red (Nacalai Tesque), washed twice, and stained with trypan blue stain (0.4%) (Life Technologies Corporation, NY, USA). The number of viable cancer cells was counted in duplicate with a Countess (Invitrogen, Carlsbad, CA, USA). The experiments were repeated more than three times.

Experimental procedures were performed in accordance with the 'Guidelines for Proper Conduct of Animal Experiments' prepared by the Science Council of Japan. The study was approved by the Animal Care and Use Committee of Kyoto Prefectural University of Medicine.

Histopathology. The peritoneal MKN45 tumor nodules cut by the USAD in the cancer cell scattering test were submitted to histopathological examination. The specimens were fixed in 4% paraformaldehyde for 24 h and embedded in paraffin. Five-micrometer-thick sections were cut, deparaffinized, rehydrated, and stained with hematoxylin and eosin.

Statistical analysis. Variables are presented as means±standard deviation and were analyzed using the Student's *t*-test. Statistical tests were two-sided, and *p*-values less than 0.05 were considered statistically significant.

Results

Existence of viable cancer cells in USAD-generated splashes and mist. The existence of viable cancer cells was confirmed *ex vivo* at 10-11 days after cell culture of mouse cancer nodules cut by the USAD in both the slow grasp group and the destructive grasp group. Representative microscopic views of each group are shown in Figure 2. A colony of the typically round cancer cells of MKN45 are evident in Figure 2B. While viable cancer cells were observed in almost all experiments, the number of viable cancer cells in the slow grasp group was much lower than that in the destructive grasp group ($4.5\pm3.5\times10^5$ *vs.* $11.5\pm3.9\times10^5$; $p=0.0357$). Viable cells were not observed in cultures of the splashes and mist harvested from the control omentum.

Histopathology of tumors cut by the USAD. When the tumors were crushed and the USAD was simultaneously activated, the cancerous tissue was exposed, but with slow grasping, and the cut surface of the tumor was observed by microscopy under the assumption that the surface was covered by thermal denaturation. The surface of cancerous tissue cut by the activated USAD was partially heat-denatured, but in other parts of the tumor, cancerous tissue was often exposed on the surface (Figure 3A). However, when tumor nodules were destructively grasped and then the USAD was activated, cancerous tissue was completely exposed on the surface (Figure 3B).

Discussion

The development of USADs brought about a revolution in gastrointestinal surgery, reducing blood loss, surgery time, and postoperative complications in gastric cancer (8, 9). However, we speculated whether viable cancer cells are included in the splashes and mist scattered from tissue as a result of USAD use during the treatment of malignant tumors. In this study, to resolve this clinical question, we examined whether USADs could cause peritoneal dissemination.

In the experiment examining oncological safety, our hypothesis was confirmed. We demonstrated the presence of viable cancer cells in the splashes and mist generated by the USAD, suggesting the possibility of iatrogenic dissemination. This event was observed in almost all experiments. Most surgeons believe that the heat from the USAD would kill any cancer cells in the mist, but the diffusion of the splashes and mist begin just after activation. However, the manner of heat elevation is linear (10) and it is unlikely that the temperature has reached a level at which the protein would be denatured at the beginning of activation. Therefore, the finding that cancer cells in the splashes and mist were viable is understandable. Nduka et al. reported that there were no airborne viable cancer cells when the USAD was in use (11). In their method, viable cancer cells were not detected in smoke plumes that were harvested through a glass tube, however, the cancer cells in the mist may have been attached and trapped on the surface of the glass tube used in their study. Alternatively, viable cancer cells may have been included only in the splashes but not in the mist. In our experiment, we captured the splashes and mist scattered around the USAD and identified viable cancer cells by *ex vivo* culture. This is a situation close to real clinical practice. When we treat advanced gastric cancer, especially in the case of obese patients or in patients who have undergone neoadjuvant chemotherapy, the tissue to be cut by the USAD is so thick that it is impossible to identify the presence of cancer. In addition, we occasionally need to cut lymphatic tissue itself; for

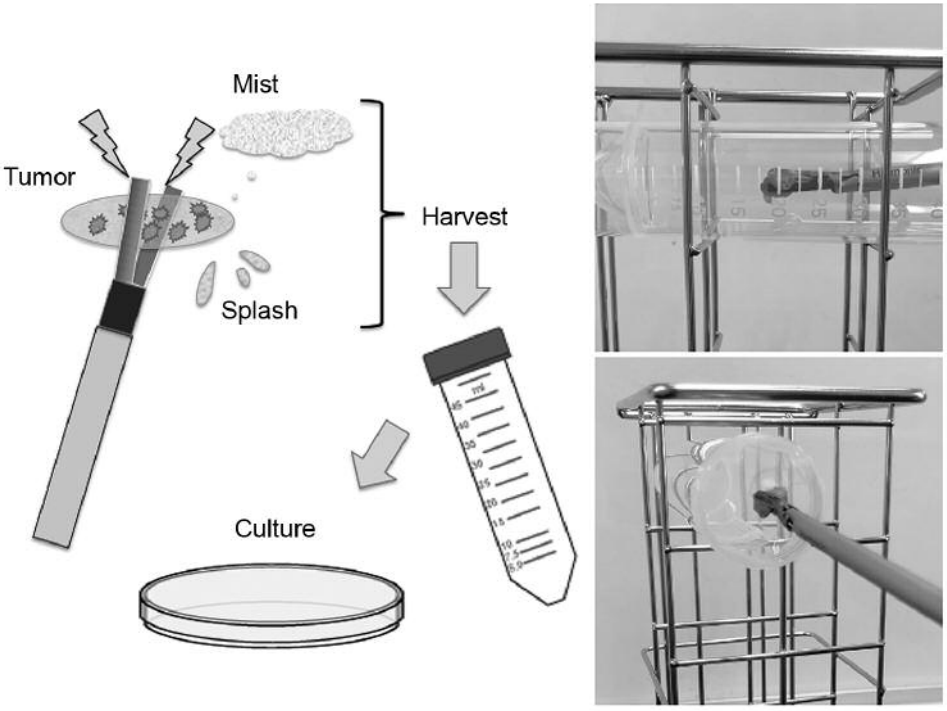
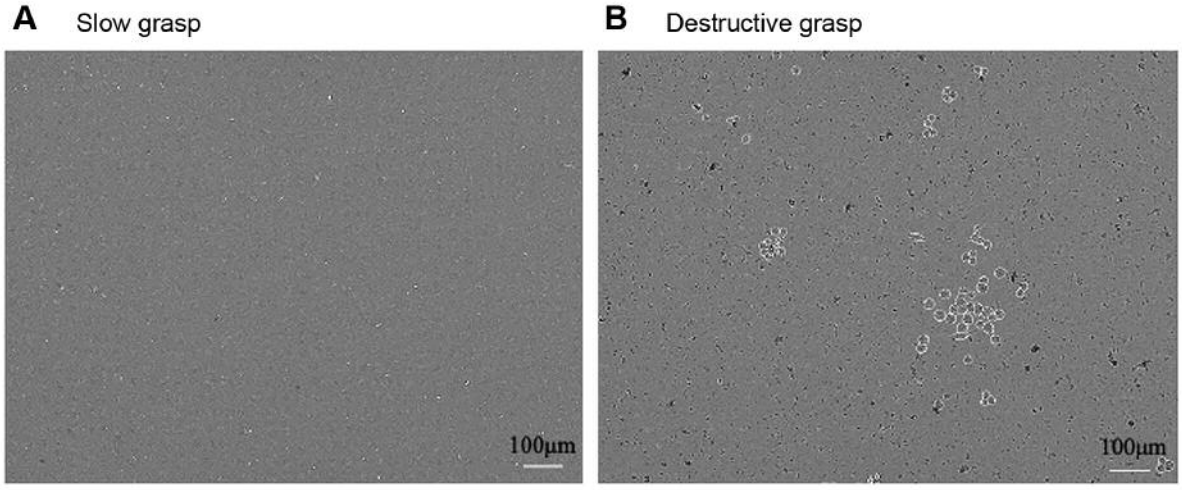


Figure 1. *Procedure of the cell scattering test. Mouse disseminated nodules were cut in a 50-ml tube by the USAD. The splashes and mist that scattered and attached on the surface of the tube were harvested and cultivated in culture media.*



Cell number 10 days after cultivation (x10^5)			*p*-Value
A 4.5±3.5	**B**	11.5±3.9	0.0357

Figure 2. *Existence of viable cancer cells. The splashes and mist were harvested and cultured in the media for 7 days. As a result, morphologically typical spheroid-shaped MKN45 cancer cells were cultured. Representative images of the ex vivo cell cultures are shown. Viable cancer cells were observed in both conditions: (A) the slow grasp group and (B) the destructive grasp group. However, the number of viable cancer cells in the slow grasp group was much lower than that in the destructive grasp group (bottom panel; p=0.0357).*

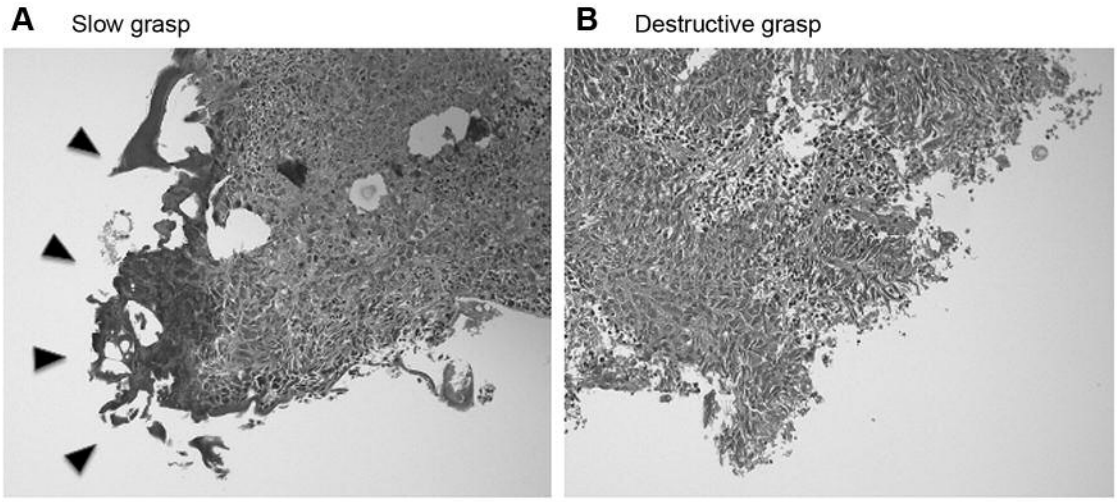


Figure 3. *Mouse cancer nodule sections cut by the USAD. Representative hematoxylin and eosin staining images of the mouse peritoneal tumor sections cut by USAD are shown. (A) The tumor nodule was slowly grasped and the USAD was simultaneously activated. The tumor was partially heat-denatured (black arrowheads), but in other parts the cancerous tissue was exposed. (B) The tumor nodule was destructively grasped, and then the USAD was activated. The cancerous tissue was completely exposed.*

example, the tissues between number 8a and 8p, or 8a and 12a lymph nodes (12). In these cases, surgeons should fully appreciate that there is the possibility that cancer cells could be spread by USAD use. However, in our experiment, cancer scattering was reduced by grasping the tumor slowly and initiating activation at the same time. This is probably because the surface of the cancerous tissue was covered by denatured proteins. However, in many cases it was imperfect and variable and may allow cancer cell spreading. Therefore, careful and precise use of USADs to avoid crushing the tissue, plus the use of vessel sealing systems, or the future development of USADs that generate less mist, are required. Most free cancer cells scattered in the peritoneal cavity will die as a result of host immune system attack, but if the patient is in an immunodeficient state or if the scattered cancer cells are caught within a defect of the peritoneal mesothelium such as a port-site, peritoneal recurrence may occur.

This study has some limitations. For cancer cell scattering tests, we demonstrated the existence of viable cancer cells in the splashes and mist generated by the USAD, but not the development of peritoneal dissemination, which varies widely because it is dependent upon the number of cancer cells, its aggressiveness, and the host immune status.

In conclusion, USADs are convenient and essential for less invasive surgeries. However, there seems to be no doubt that viable cancer cells are scattered when cutting cancerous tissue using USADs. Surgeons should fully understand the characteristics of USADs and pay careful attention to cancer cell dissemination.

Conflicts of Interest

The Authors declare no conflicts of interest related to this study.

Authors' Contributions

T.K. conceived of the presented idea and planned the experiments. S.K., T.O., T.K., H.K., A.S., H.F. and K.O., who are also members of the Upper GI team of our Department, encouraged T.K. to investigate and gave some helpful comments to this work. T.K., K.S., S.O., T.M., H.K. and M.Y. carried out the experiments. T.A., H.S., Y.Y., R.M., H.I. and Y.K. contributed to the interpretation of the results. E.O. is managing our Department and supervised the work. All Authors discussed the results and contributed to the final manuscript.

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References

1 Amaral JF: Ultrasonic dissection. Surg Allied Tech *2(3-4)*: 181-185, 1994. PMID: 8000888.

2 Foschi D, Cellerino P, Corsi F, Taidelli T, Morandi E, Rizzi A and Trabucchi E: The mechanisms of blood vessel closure in humans by the application of ultrasonic energy. Surg Endosc *16(5)*: 814-819, 2002. PMID: 11997829. DOI: 10.1007/s00464-001-9074-x

3 Berends FJ, Kazemier G, Bonjer HJ and Lange JF: Subcutaneous metastasis after laparoscopic colectomy. Lancet

344(8914): 58, 1994. PMID: 7912321. DOI: 10.1016/s0140-6736(94)91079-0
4 Paik PS and Beart RW: Laparoscopic colectomy. Surg Clin North Am *77(1)*: 1-13, 1997. PMID: 9092105. DOI: 10.1016/s0039-6109(05)70529-6
5 Liang Y, Li G, Chen P and Yu J: Laparoscopic *versus* open colorectal resection for cancer: A meta-analysis of results of randomized controlled trials on recurrence. Eur J Surg Oncol *34(11)*: 1217-1224, 2008. PMID: 18155389. DOI: 10.1016/j.ejso.2007.11.004
6 Liang Y, Li G, Chen P, Yu J. Laparoscopic *versus* open colorectal resection for cancer: a meta-analysis of results of randomized controlled trials on recurrence. Eur J Surg Oncol *34(11)*: 1217-1224, 2008. PMID: 18155389. DOI: 10.1016/j.ejso.2007.11.004
7 Zanghi A, Cavallaro A, Piccolo G, Fisichella R, Di Vita M, Spartà D, Zanghi G, Berretta S, Palermo F and Cappellani A: Dissemination metastasis after laparoscopic colorectal surgery *versus* conventional open surgery for colorectal cancer: a metanalysis. Eur Rev Med Pharmacol Sci *17(9)*: 1174-1184, 2013. PMID: 23690186.
8 Tsimoyiannis EC, Jabarin M, Tsimoyiannis JC, Betzios JP, Tsilikatis C and Glantzounis G: Ultrasonically activated shears in extended lymphadenectomy for gastric cancer. World J Surg *26(2)*: 158-161, 2002. PMID: 11865342. DOI: 10.1007/s00268-001-0199-9
9 Inoue K, Nakane Y, Michiura T, Yamada M, Mukaide H, Fukui J, Miki H, Ueyama Y, Nakatake R, Tokuhara K, Iwamoto S, Yanagimoto H, Toyokawa H, Satoi S and Kwon AH: Ultrasonic scalpel for gastric cancer surgery: a prospective randomized study. J Gastrointest Surg *16(10)*: 1840-1846, 2012. PMID: 22833440. DOI: 10.1007/s11605-012-1970-y
10 Hayami M, Watanabe M, Mine S, Imamura Y, Okamura A, Yuda M, Yamashita K, Toihata T, Shoji Y and Ishizuka N: Lateral thermal spread induced by energy devices: a porcine model to evaluate the influence on the recurrent laryngeal nerve. Surg Endosc *33(12)*: 4153-4163, 2019. PMID: 30847557. DOI: 10.1007/s00464-019-06724-y
11 Nduka CC, Poland N, Kennedy M, Dye J and Darzi A: Does the ultrasonically activated scalpel release viable airborne cancer cell? Surg Endosc *12(8)*: 1031-1034, 1998. PMID: 9685536. DOI: 10.1007/s004649900774
12 Japanese Gastric Cancer Association. Japanese classification of gastric carcinoma: 3rd English edition. Gastric Cancer *14(2)*: 104-105, 2011. PMID: 21573743. DOI: 10.1007/s10120-011-0041-5

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