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## Abstract

*Introduction*: Avoiding tissue desiccation is a common recommendation to reduce postoperative complications following open abdominal surgery, although difficult to achieve delicately without damaging the peritoneal mesothelium. Insufflation of humidified-warm $CO_2$ into the abdomen during open abdominal surgery is proposed as an invisible, effortless way to prevent desiccation. We hypothesized that desiccation during open abdominal surgery would cause loss of peritoneal mesothelium that would be prevented by insufflation of humidified-warm $CO_2$.

*Methods*: Nine Wistar rats were assigned to 1 h of anesthesia only, laparotomy only, or laparotomy with insufflation of humidified-warm $CO^2$. Twelve hours after treatment, rats were euthanized and tissue samples were excised. Scanning electron microscopy (SEM) and light microscopy (LM) images of visceral and parietal peritoneum were scored by two independent, blinded examiners for loss of mesothelium and other indications of inflammation, including measurement of apoptosis by detection of DNA cleavage.

*Results*: Loss of peritoneal mesothelium was found in peritoneum exposed to laparotomy only (SEM: $P = 0.002$; LM: $P = 0.01$), and mesothelial loss was reduced by humidified-warm $CO_2$ (SEM: $P < 0.001$; LM $P = 0.004$). Similarly, DNA cleavage was significantly higher on the peritoneal surface following laparotomy only, compared with anesthesia only ($P = 0.0055$) and laparotomy with humidified-warm $CO_2$ insufflation ($P = 0.0003$).

*Conclusions*: In a rat model, exposing the peritoneal mesothelial to conditions that replicate minimum recommended air flow within an operating room causes inadvertent loss of mesothelium and signs of inflammation that can be prevented by insufflating humidified-warm $CO_2$ into the open abdominal cavity.



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# Laparotomy causes loss of peritoneal mesothelium prevented by humidified $CO_2$ insufflation, in rats

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ABSTRACT

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*Conclusions:* In a rat model, exposing the peritoneal mesothelial to conditions that replicate minimum recommended air flow within an operating room causes inadvertent loss of mesothelium and signs of inflammation that can be prevented by insufflating humidified-warm $CO_2$ into the open abdominal cavity.



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## Introduction

The peritoneal mesothelium plays an essential role in the prevention of postoperative complications including adhesion formation and peritoneal tumor implantation. Five percent of patients will have at least one readmission directly related to adhesions within 5 y of colorectal surgery, excluding appendectomies.[1] Over a 10-year period in the UK, an estimated 908 million Euros is spent on adhesion-related readmissions following lower abdominal surgery.[2]

Following damage to the peritoneal mesothelium, as inevitably occurs during surgical incision, the presence of neighboring mesothelial cells is essential to control the delicate balance between the deposition and breakdown of fibrin and to allow the mesothelium to heal adhesion free.[3-5] In the event of intraperitoneal tumor spillage, mesothelial cells are required to secrete free hyaluronic acid to bind to intraperitoneal tumor cells, inhibiting them from adhering and thereby metastasizing to the peritoneum.[5] In addition, it has been shown that tumor cells adhere preferentially to areas where the mesothelium is disrupted in acute *in vitro* human[6] and animal studies,[7,8] and in tissue culture investigations.[9]

One cause of inadvertent loss of peritoneal mesothelium is desiccation.[10-17] Desiccation is traditionally reduced using irrigating lavage and by placing wet packs into the abdominal cavity. However, criticism is growing against the unnecessary use of intraperitoneal lavage, as it may increase the risk of postoperative complications by disrupting the peritoneal mesothelium, and it is not effective in reducing the risk of surgical site infection.[3,5,18] Furthermore, it is likely that rubbing the peritoneum with a wet pack can also cause mesothelial damage.[11,19]

Insufflation of humidified-warm carbon dioxide ($CO_2$) into the abdominal cavity has been proposed as a therapy to reduce inadvertent damage to the peritoneal morphology caused by desiccation during open abdominal surgery.[20] Using an active humidification system and a specially designed gas diffuser, humidified-warm $CO_2$ can be diffused into the open peritoneal cavity at a low velocity while at a flow rate high enough to create a local environment with a high concentration of $CO_2$.[21] An invisible humidified greenhouse effect is created within the open abdominal cavity that improves tissue oxygenation[22] and reduces desiccation almost completely.[23] Clinical trials have shown that the open abdominal wound remains warmer and the risk of hypothermia is reduced during surgery,[24,25] and surgical costs are reduced.[26]

Following endoscopic surgery, reduction of desiccation by insufflating humidified-warm $CO_2$ has been shown to reduce mesothelial cell loss and inflammatory changes.[13,27-30] Therefore, it has been hypothesized that the use of humidified-warm $CO_2$ will also reduce loss of peritoneal mesothelium in open abdominal surgery.[31] Furthermore, despite the evidence that desiccation causes loss of mesothelium, there is a lack of evidence as to whether exposure of the mesothelium to the ambient operating room air ventilation during open abdominal surgery without $CO_2$ insufflation causes sufficient desiccation to result in mesothelial cell loss. In addition, investigations during laparoscopy suggest that loss of mesothelium will be by apoptosis,[32] will be proceeded by a change in parietal cellular morphology from a flat to relatively bugled cell,[27,29,33] and will increase the expression of the inflammatory marker COX-2 that is an important predictor of cancer progression.[14] It is also likely that the inflammation will extend to portions of the peritoneum that are not exposed to the desiccating environment and that submesothelial edema will occur.[34]

This research was designed to test two primary hypotheses. First, that exposure of the peritoneal mesothelial to normal operating room air ventilation during open abdominal surgery will cause inadvertent loss of peritoneal mesothelial cells compared with anesthesia only controls. Second, that insufflation of humidified-warm $CO_2$ into the open abdominal cavity will reduce the loss of peritoneal mesothelial cells compared with laparotomy without gas insufflation. Data were also collected to explore the hypotheses that laparotomy without gas insufflation, compared with both surgery with insufflation of humidified-warm $CO_2$ and anesthesia only controls, will cause bulging of parietal mesothelial cells, increased expression of the inflammatory marker COX-2, increased submesothelial cell thickness; and apoptosis.

## Materials and methods

This study was approved by the University of Wollongong Animal Ethics Committee (AE 10-24). Nine female Wistar rats were used accordance with the Australian Code for the Care and Use of Animals for Scientific Purposes.[35] Before surgery, the rats were housed two rats to a cage with ad libitum access to food and water. The rats were maintained in a temperature controlled environment with diurnal variation of light.

The animal experimental setup has previously been described in detail.[22]

Prophylactic pain relief was administered (subcutaneous meloxicam 1 mg/kg). Core body temperature was monitored every 5-10 min with a rectal thermometer. Insensible fluid replacement was delivered hourly at 10 mL/kg/h subcutaneously with warmed 0.9% sodium chloride, according to Australian guidelines for the promotion of well-being of animals used for scientific purposes.[36]

Rats were assigned to one of three groups.

1. Group C: anesthesia only control ($n = 2$)
2. Group LO: laparotomy only with controlled ambient air flow ($n = 4$)
3. Group LI: laparotomy with insufflation of humidified-warm $CO_2$ ($n = 3$)

Following commencement of mechanical ventilation, the abdomen was clipped and cleaned. In groups LO and LI, an inverted "L" shaped laparotomy incision (60-mm long midline incision, starting 10 mm caudal to the xiphoid process, and a 40 mm long incision across the left side of the abdominal wall, extending from the rostral end of the first incision). The abdominal wall was then gently reflected toward the lower left quadrant to expose the parietal peritoneum. The skin was

clamped to minimize tension on the peritoneum. To further expose the parietal peritoneum, the left hind leg was flexed and secured using tape across the paw. To expose the spleen, the spleen was gently moved anteriorly with forceps applied to the underlying connective tissue to ensure that the mesothelial surface was untouched. The relevant treatment condition was then applied for 1 h.

In group LO, to mimic conservative operating room air ventilation, a Perspex chamber (460 × 460 mm square, height 480 mm) was placed over the rat. A small fan in the ceiling of the chamber was calibrated to create 20 exchanges of the chamber air per hour,[37,38] with air exiting through a 3 mm gap at the chamber base.

In group LI, the rat was placed in a 7 L plastic container (355 × 235 mm, height 120 mm) with a 9 × 12 cm hole in the top through which the $CO_2$ was insufflated, so as to ensure the abdominal cavity, relatively shallow in the rat model, was exposed to a stable high concentration of $CO_2$. The $CO_2$ was continuously insufflated into the container at 9 L/min via a gas diffuser (VitaDiffuser, Cardia Innovation, Sweden). Pilot measurements of $CO_2$ concentration using a CheckMate II gas analyzer (PBI Dansensor, Denmark) showed that the environment within the box is maintained at > 90% $CO_2$. The ability to create an environment of high $CO_2$ concentration within a surgical cavity has been well documented.[21,39,40] The $CO_2$ was humidified and warmed using a humidifier controller and delivered to the gas diffuser by a heated delivery tube (HumiGard, Fisher and Paykel Healthcare, New Zealand). Independent testing has shown that the humidifier delivers >98.0% relative humidity at 37°C.[41]

Following treatment, the peritoneum was sutured closed, the skin was stapled, and a bandage was applied to protect the wound. The rat was then returned to an individual cage after surgery and monitored for signs of pain. An extra dose of pain relief was administered if necessary (subcutaneous meloxicam 1 mg/kg).

Twelve hours after treatment, rats were euthanized by $CO_2$ asphyxiation as this has previously been shown to be the time of maximum mesothelial inflammation.[8,33] Tissues of the left abdominal wall only (control group) or both abdominal walls (LO and LI groups) and spleen (all groups) were excised and pinned out in 100 mmol/L sodium phosphate buffer with 2% sucrose, pH 7.3 (buffer).[11] Following rinsing with buffer, the buffer was replaced with Bouin's fixative[42] containing an additional 0.2% glutaraldehyde, and left for 36 h and then stored in buffer at 4°C. Each abdominal wall was then divided into three portions. Two 5 $mm^2$ samples were then taken from each portion, and from the spleen. One sample from each portion of tissue was used for paraffin embedding and the other for preparation for scanning electron microscopy (SEM) analysis. All images were captured by researchers blinded to the group allocation of the samples. All analysis was carried out by two independent and blinded evaluators, except relatively objective measures of submesothelial thickness and average fluorescence that were analyzed by one blinded evaluator.

Tissue samples for paraffin embedding were dehydrated in graded alcohol solution, embedded in paraffin taking care to mount the tissue parallel to the face of the wax block and sectioned to 5 μm.[27,29]

### *Scanning electron microscopy preparation and analysis*

Before SEM analysis, tissue samples were dehydrated in graded ethanol solutions, immersed in 100% hexamethyldisilazane, and air dried in a sealed container with a desiccant for at least 2 d before mounted onto stubs and sputter coated with gold. Two areas of interest were chosen using a template that was placed over the low magnification image to avoid bias, and imaged at 2000×, 650×, and 300× (JEOL JSM-6490LV, JEOL Ltd, Tokyo, Japan). All images were evaluated for area percentage of mesothelial cell loss, using a previously published stereology method,[43] and mesothelial cell bulging. Mesothelial cell bulging was evaluated on a semiquantitative three level scale, similar to previous publications,[29] in which 0 = none or slight, 1 = moderate (20%-39% of surface affected), and 2 = marked (40%-100% of surface affected).

### *Analysis of hematoxylin and eosin staining sections by light microscopy*

Light microscopy imaging was conducted at 20× magnification (Leica DM6000, Leica microsystems, Wetzlar, Germany) and a mosaic was created, allowing analysis of the entire width of the tissue section. Mesothelial cell loss and bulging was then evaluated on a semiquantitative three-level scale, as described for SEM. The thickness of submesothelial connective tissue was measured perpendicular to the peritoneal surface[44] in the widest portion cut parallel to the underlying muscle fibers[45] using the software package LAS, v 4.3 (Leica Microsystems, Wetzlar, Germany) at 322× magnification.

### *Detection of apoptosis by TUNEL assay*

Detection of apoptotic mesothelial cells on exposed peritoneum was carried out by labeling cleaved DNA using a terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) assay (In Situ Cell Death Detection Kit TMR red, Roche Applied Science, Basel, Switzerland), as per the kit instructions. DNA cleavage was induced in positive controls using DNase I (Roche Diagnostics, Basel, Switzerland).

Average fluorescence along the peritoneal surface was quantified in scanned sections (Aperio FL Multiplexing Immunofluorescence Slide Scanner, Leica Biosystems, Nussloch, Germany; excitation wavelength 590, detection wavelength 617 nm) using Image J (free software available at http://fiji.sc/Fiji) without any image processing. Background subtraction was carried out using average fluorescence in adjacent muscle fibers for abdominal wall tissue or white pulp for spleen tissue. Average fluorescence was then expressed as a percentage of the average background fluorescence for each section.

### *Analysis of COX-2 expression by immunohistochemistry*

Detection of COX-2 expression in exposed tissue by immunohistochemistry was carried out as previously described.[14] Four representative images were taken at 50x magnification from each sample (Leica DM6000 optical microscope, Lecia microsystems, Wetzlar, Germany). Images were scored for intensity of staining (0 = none; 1 = weak;

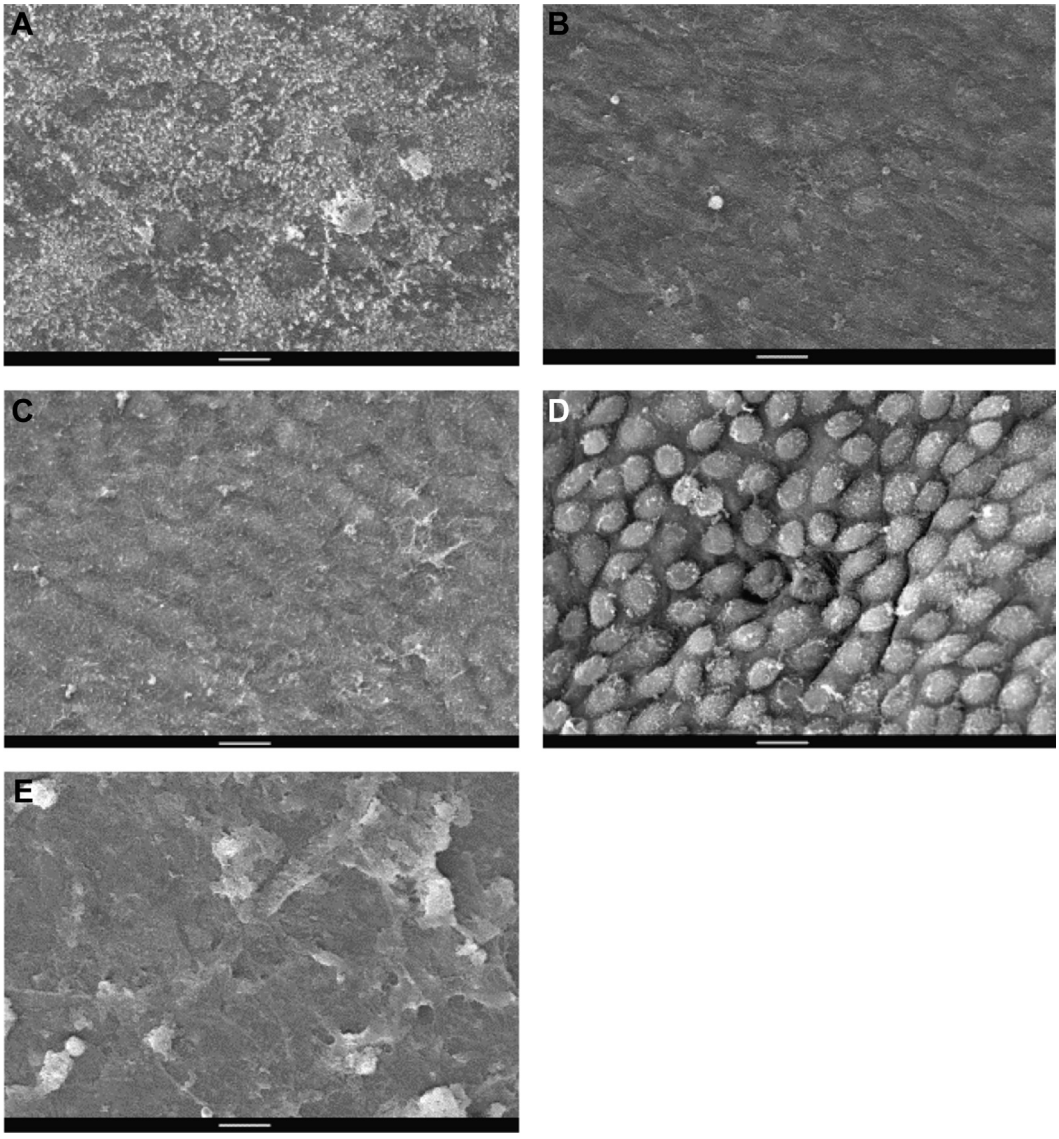


**Fig. 1 – Examples of mesothelial changes observed by SEM. (A) Control group, continuous coverage with flat mesothelial cells, identified by the presence of microvilli and cell borders. (B) LI exposed, continuous coverage with flat mesothelial cells. (C) LO nonexposed, continuous coverage with mesothelial cells, moderate bulging. (D) LO nonexposed, marked bulging. (E) LO exposed, massive loss of mesothelial cells and exposure of the underlying basement memebrane. (Bar = 20 μm). LI = laparotomy with insufflation; LO = laparotomy only.**

2 = medium; and 3 = strong), similar to a previously published method.[14] The scores given to each of the four images from each section were averaged to give one score per tissue section.

### *Statistical analysis*

The scores of the two evaluators were averaged to give a score for each image. A Shapiro-Wilk normality test was used to test for normality. When a difference between groups was detected using an independent samples ANOVA or Kruskal-Wallis test, an independent samples *t*-test or Mann-Whitney test was used to determine whether differences between individual groups were present. *P* value $< 0.05$ was considered statistically significant. Outcomes were reported as mean (standard deviation) or as median (25th-75th percentile).

## Results

The average weight of the rats was 314 g (standard deviation = 58), and did not differ between groups ($P < 0.05$ for all comparisons). Examples of mesothelial changes observed are shown in Figures 1 and 2.

In peritoneum from the abdominal wall exposed to the gaseous environments, mesothelial cell loss in the LO group was larger than both group C and LI, see Figure 3. This pattern of loss of mesothelium was seen in exposed tissue analyzed

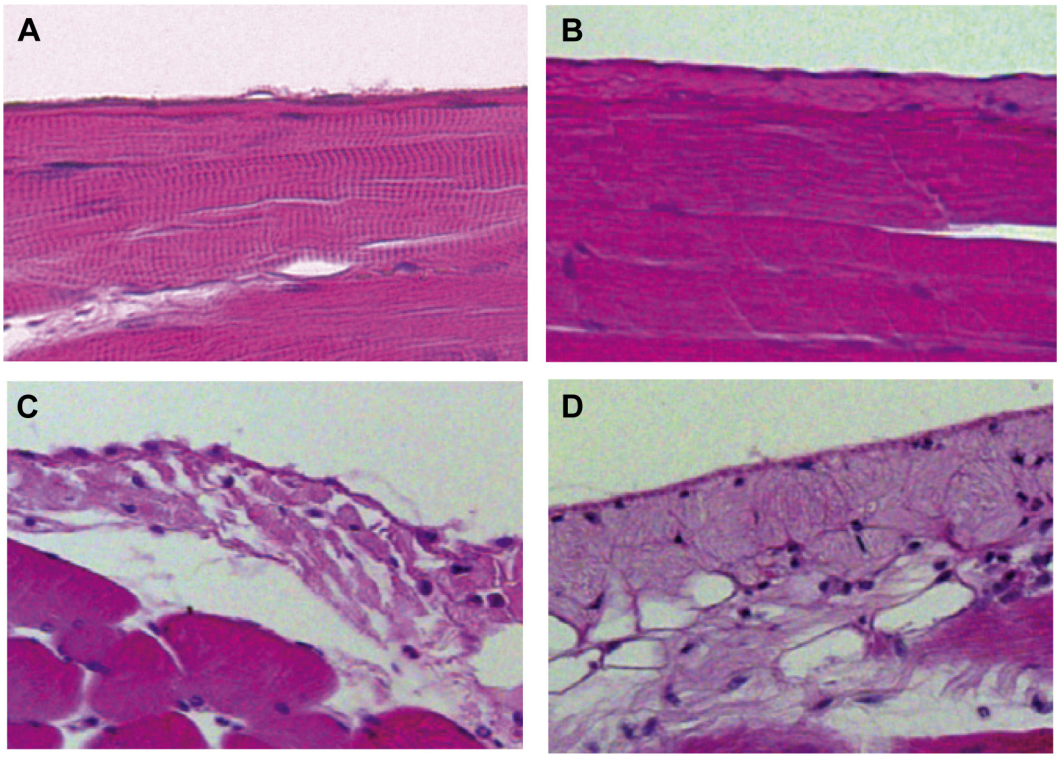


**Fig. 2 – Examples of changes observed in light microscopy images (imaged at 20× magnification). (A) Control group, a continuous layer of flat mesothelial cells. (B) LI exposed, a continuous layer of flat mesothelial cells. (C) LO exposed, bulging of mesothelial cells and increase in submesothelial thickness. (D) LO exposed, total loss of mesothelium and increase in submesothelial thickness. C = control, anesthesia only; LO = laparotomy only; LI = laparotomy with insufflation. (Color version of figure is available online.)**

by both scanning electron and light microscopy. There was very little mesothelial cell loss seen in nonexposed peritoneum (see Figure 4).

Adherent mesothelial cells of the parietal peritoneum scored from SEM images showed significantly greater cellular bulging in the LO group (0.5, 0-1.5) compared with both group C (0, 0-0.38), $P = 0.026$, and LI (0, 0-0.5), $P = 0.028$. Bulging of the parietal mesothelium was not detected in tissue sections analyzed by light microscopy analysis in either exposed or nonexposed samples (C 0, 0-0; LI exposed 0, 0-0; LO exposed 0, 0-0.5; LI nonexposed 0, 0-0.5; LO nonexposed 0, 0-0.25; all $P$ values $> 0.05$).

Submesothelial thickness of the LO group (34.19, 28.07-58.06 μm) was higher than both the C (16.46, 4.825-20.49 μm, $P = 0.0182$) and LI (17.83, 13.42-25.26 μm, $P = 0.0012$) groups. There was no significant difference between exposed and nonexposed tissue measurements, so those groups were combined (LO exposed *versus* nonexposed $P = 0.4290$; LI exposed *versus* nonexposed $P = 0.4103$). COX-2 expression was not significantly different between any of the groups, Kruskal-Wallis test (C 2, 1.2-2.5; LO 1.8, 1.5-2.3; LI 1.8, 1.2-1.9; and $P = 0.46$).

Representative results of the TUNEL assay for detection of apoptosis are shown in Figures 5 and 6. Quantification showed significantly higher average fluorescence on the surface of exposed peritoneum in the LO group (69.0, 10.5%-151.5%) compared with both group C (1.5, −9.75% to 8.0%, $P = 0.0055$) and LI (−20.0, −41.0 to 6.0 %, $P = 0.0003$). In the spleen tissue samples, comparison with adjacent and nonexposed peritoneum was possible. Red fluorescence was observed on the exposed surface that was visible at low magnification. The fluorescence extended beneath the mesothelium and was not seen on the nonexposed surfaces, see Figure 6.

## Discussion

This study has, to our knowledge, for the first time established that simply exposing the peritoneum to conditions that replicate recommended air flow within an operating room causes sufficient desiccation to result in inadvertent loss of parts of the mesothelium. The mesothelial loss was consistent in peritoneal tissue independently analyzed by both scanning electron and light microscopy. An extraordinary 25% of the peritoneum sampled had more than half its surface area desquamated of mesothelial cells. Furthermore, significant loss of mesothelium was only observed on the peritoneum exposed to the air flow and not on the contralateral nonexposed abdominal wall. This suggests that the inadvertent loss of mesothelium is caused by exposure to the air flow, rather than the large surgical incisions made in the abdominal wall. Evidence was also found to support the second hypothesis that inadvertent loss of mesothelium by desiccation can be prevented with

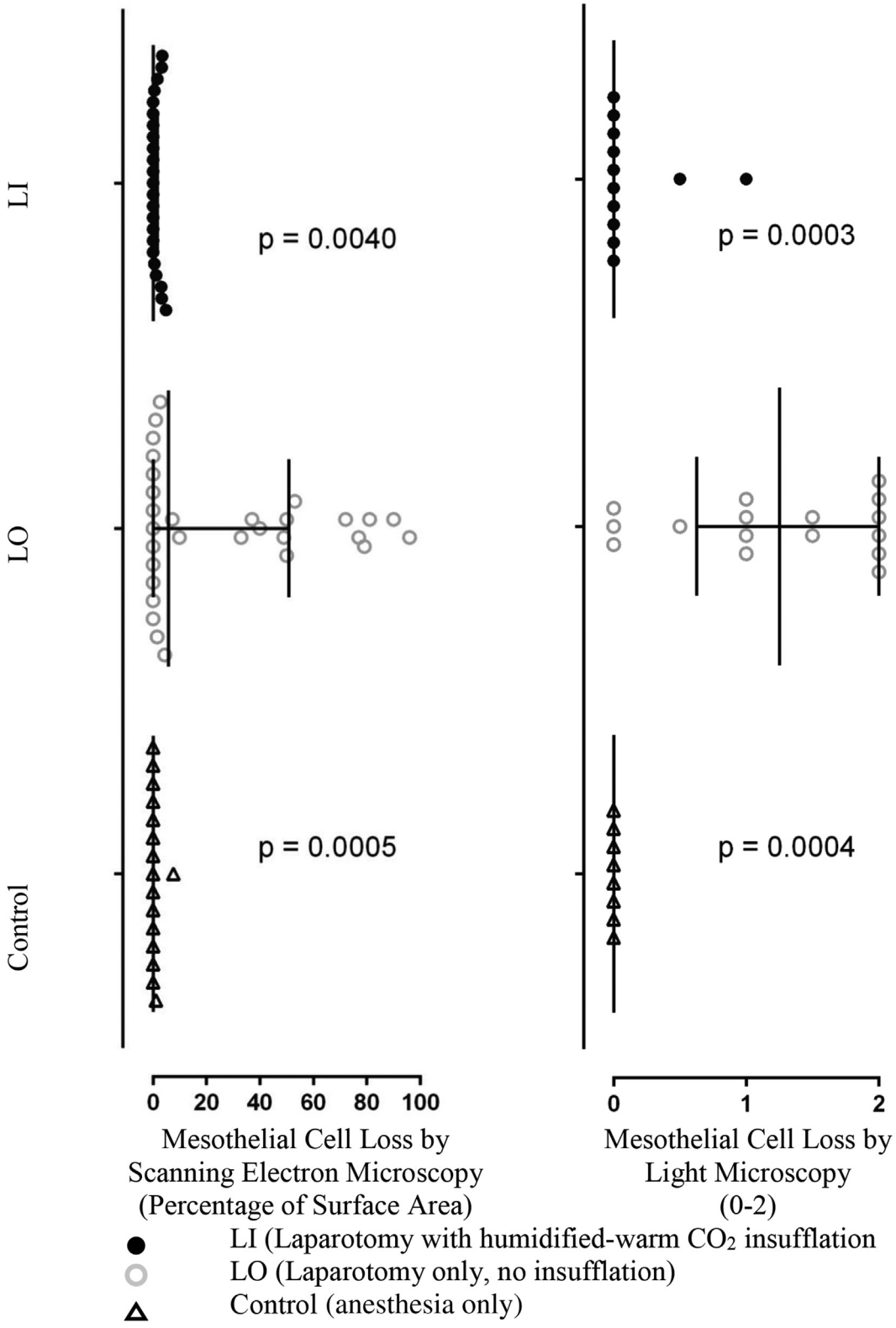


**Fig. 3 – Results of analysis of mesothelial cell loss of the exposed peritoneum and anesthesia only controls. Left: Scanning electron microscopy analysis showed the area percentage of mesothelial cell loss from peritoneum exposed to the gaseous environment in the LO group (5.8, 0%-50.8%) was larger than both group C (0, 0%-0%, $P = 0.0005$), and LI (0, 0%-1.7%, $P = 0.0040$). Right: Semiquantitative analysis by light microscopy showed loss in the LO group (1.25, 0.625%-2%) was larger than both group C (0, 0%-0%, $P = 0.0004$) and group LI (0, 0%-0%, $P = 0.0003$).**

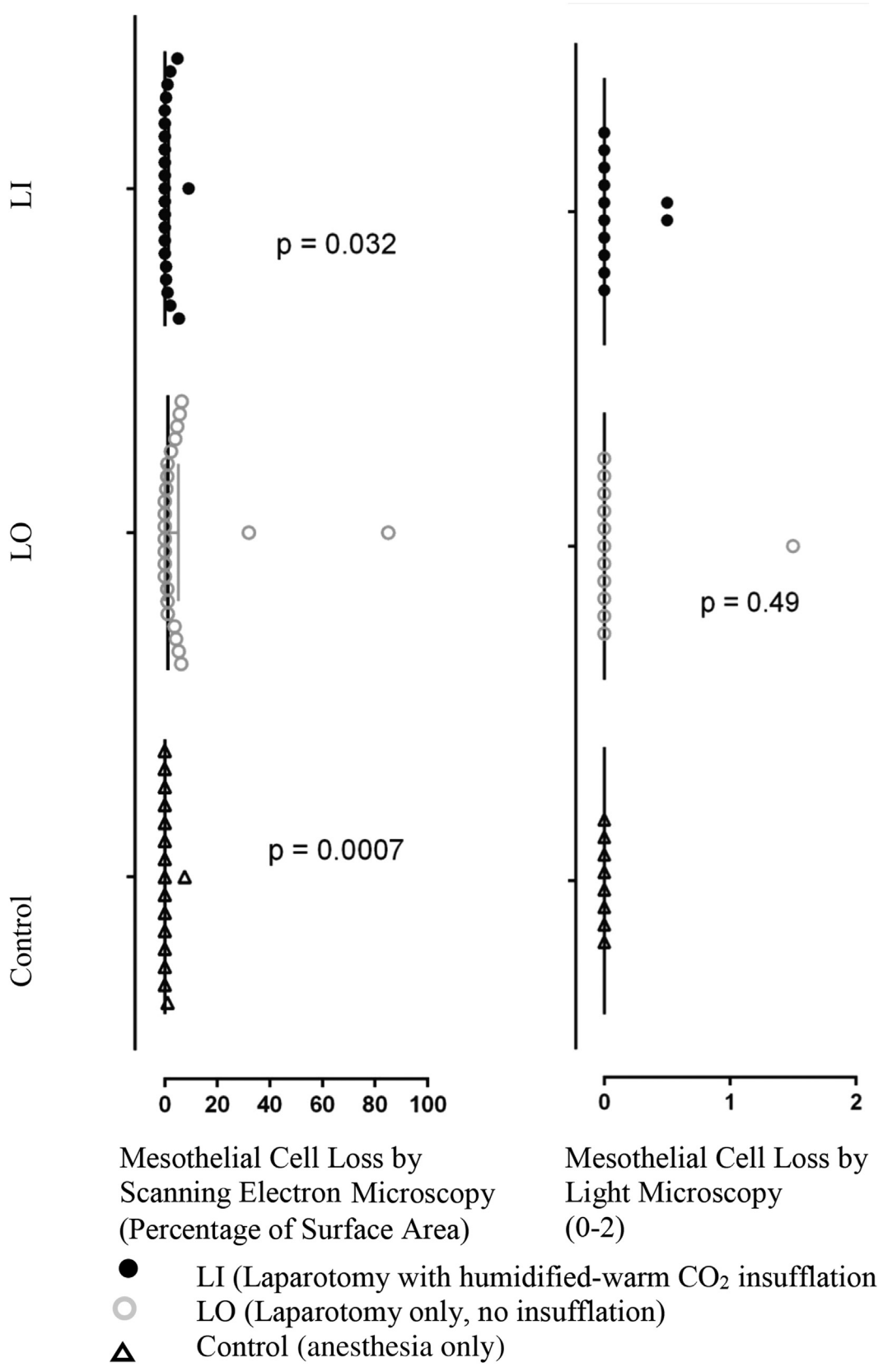


**Fig. 4 – Results of analysis of mesothelial cell loss of the nonexposed peritoneum and anesthesia only controls. Left: SEM, the area percentage of mesothelial loss in the LO group (1.2, 0%-5.2%), was slightly larger than the group C (0, 0-0), $P = 0.0007$, and LI groups (0, 0%-1.35%, $P = 0.032$). Right: No significant differences in mesothelial cell loss was seen by light microscopy analysis ($P = 0.49$).**

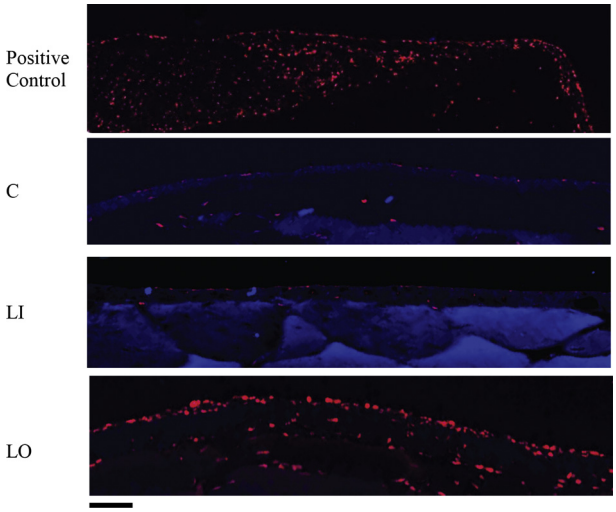


**Fig. 5 – Example fluorescent microscopy images of parietal peritoneal tissue stained with a TUNEL assay for the detection of apoptosis. C and LI: Little fluorescent red TUNEL staining along the peritoneal edge. LO: Significantly increased fluorescent red TUNEL staining along the peritoneal edge. Bar = 50 μm. Blue coloring is including to allow visualization of adjacent tissue structures and is due to technical difficulties with DAPI staining, likely due to the use of glutaraldehyde fixation. C = control, anesthesia only; LO = laparotomy only; LI = laparotomy with insufflation. (Color version of figure is available online.)**

humidified-warm $CO_2$ insufflation into the open abdominal cavity.

Furthermore, the results suggest that this loss of mesothelium following laparotomy was accompanied by signs of inflammation in the mesothelial cells that remain adherent to the peritoneum, illustrated by increased bulging of mesothelium both on exposed peritoneum and peritoneum that was not exposed, and also an increase in submesothelial thickness following laparotomy. Humidified-warm $CO_2$ insufflation significantly reduced bulging of mesothelial cells and submesothelial thickness. However, the expression of the inflammatory marker COX-2 was the same in all groups. Unexpectedly, COX-2 expression was high also in the control group. This may have been related to administration of a nonsteroidal anti-inflammatory drug, which can unexpectedly increase COX-2 expression in some tissues.[46]

Peritoneal damage was further illustrated by a marked increase in apoptosis, measured by DNA fragmentation, on the surface of exposed peritoneum in the LO group. It is therefore probable that some of the remaining mesothelial cells in the laparotomy group were undergoing apoptosis, and quantification of mesothelial cell loss would have increased if tissue samples were taken at a later time point. In addition to fluorescence on the surface of the peritoneum, a wide band of DNA fragmentation was observed underlying the exposed surface of the spleen that was in striking contrast to the adjacent nonexposed surfaces. This observation suggests that apoptosis caused by desiccation may not be limited to the mesothelium as hypothesized, and damage may also occur in the underlying parenchymal tissue. Humidified-warm $CO_2$ insufflation significantly reduced DNA fragmentation, supporting the hypothesis that mesothelial cells that remain adherent in the LI group are not undergoing apoptosis.

The observed loss of peritoneal mesothelial cells following exposure to controlled air flow during laparotomy is consistent with previous investigations into the effect of desiccation. Experimental damage to the peritoneum, to study mesothelial healing and adhesion formation, has previously been induced with a "gentle stream"[15,16] and 30 L/min[17] of dry, compressed air for just 5 min. Furthermore, the observed results are consistent with animal models of endoscopic surgery that have shown that the loss of mesothelium by exposure to dry $CO_2$ can be reduced by humidification of the $CO_2$ gas.[13,27-30] The observed increase in submesothelial thickness is consistent with reports of submesothelial edema following desiccation of the peritoneum in laparoscopic surgery[30] and following experimentally induced injury to the mesothelium in a murine model.[34] It may be possible that an even larger increase in submesothelial thickness would be seen if the tissue samples were taken later than 12 h following surgery.[34]

Damage to the peritoneum during laparotomy is inevitable as often large surgical incisions are required and organs must be manipulated to achieve the surgical objective. However, the current research shows that additional, inadvertent damage to the parietal and visceral mesothelium is caused by desiccation simply by opening and exposing the abdominal cavity. This may have important clinical implications as uninjured mesothelial cells surrounding surgically damaged mesothelium are essential for adhesion-free healing and prevention of peritoneal tumor implantation.[3-5,34] Surgical practice has long recognized the need to prevent intra-abdominal desiccation.[47-50] However, surgeons are faced with the problem of how to prevent damage to the peritoneal mesothelium caused by desiccation during a time of growing criticism against the unnecessary use of intraperitoneal lavage,[3,5,18,47] and the knowledge that rubbing the delicate peritoneum with wet packs likely also causes mesothelial damage.[11,19] The innovation of intra-abdominal insufflation of humidified-warm $CO_2$ is that it reduces desiccation invisibly, with no effort from the surgeon and without the risks to the mesothelium associated with unnecessary intraperitoneal lavage or rubbing the peritoneum with wet packs. Insufflation of humidified-warm $CO_2$ is clinically simple and has shown to be clinically effective in reducing temperature loss during surgery in a number of randomized control trials.[24,25]

There are a number of limitations within our study. First, this study is limited by the use of an animal model. The current research design would be difficult to repeat in human subjects due to the need to delay collection of peritoneal tissue samples after the completion of surgery to allow for inflammatory to changes to occur, which is likely unethical in humans. Research in laparoscopy suggests that tissue samples taken at the time of surgery will show an intact mesothelium under SEM, even under conditions that have been show to result in cell loss when sample collection is delayed.[33] However, a recent study has used transmission electron microscopy to show that apoptotic bodies are present in human mesothelial cells of peritoneum taken at the end of laparoscopic surgery, supporting previous animal results.[51]

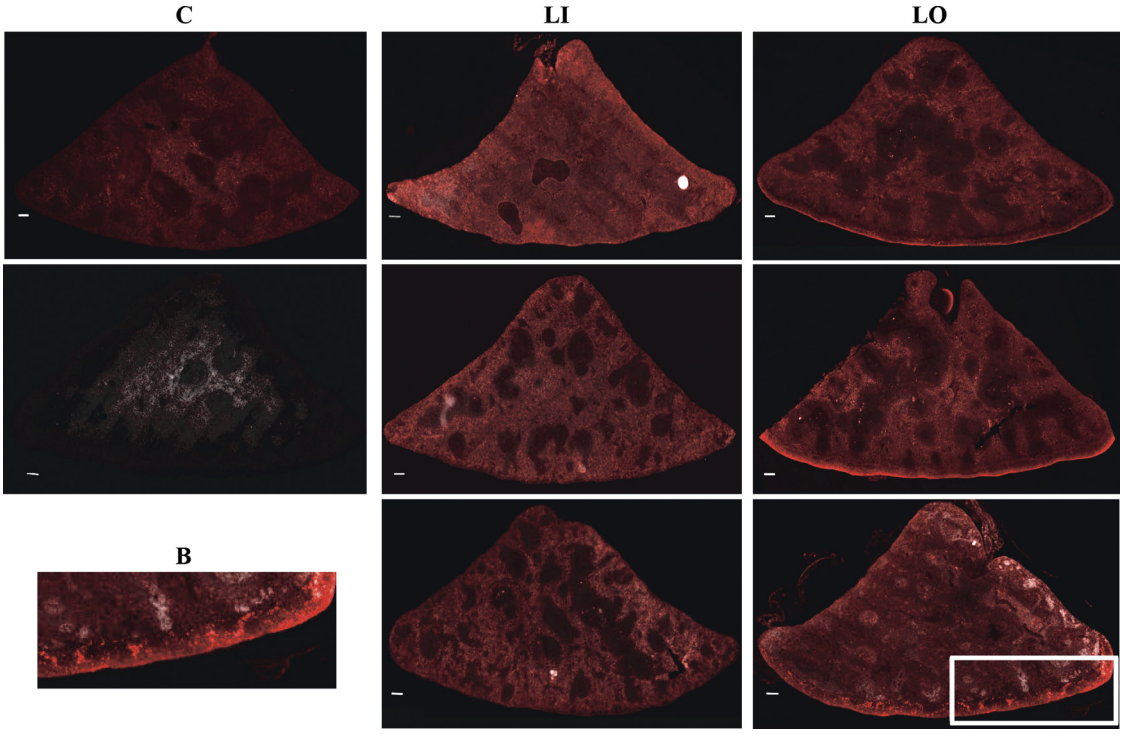


**Fig. 6 – Fluorescent microscopy images of visceral peritoneal tissue stained with a TUNEL assay for the detection of apoptosis. In all LI and LO images, the lower, convex surface of the spleen was exposed during surgery. (A) C and LI: Little fluorescent red TUNEL staining along all peritoneal edges. LO: Increase in fluorescent red TUNEL staining on the exposed peritoneal surface that is not observed on the nonexposed surfaces. Staining extends well below the mesothelium. (Note: one tissue sample from the LO group was discarded due to massive damage during processing). White bar = 200 μm. (B) An enlarged view of fluorescence present in the white box of the third LO tissue sample. C = control, anesthesia only; LO = laparotomy only; LI = laparotomy with insufflation. (Color version of figure is available online.)**

The current study was also limited by subjective identification of mesothelial cells. However, validity of cell identification is supported by the consistent results observed across independent analysis by SEM, light microscopy, and detection of apoptosis. In addition, a potential perceived limitation of the current study is that the method of insufflation of $CO_2$ was modified from clinical practice for the current model. Clinically, humidified-warm $CO_2$ is continuously insufflated directly into the abdominal cavity via a gas diffuser. $CO_2$ fills the cavity and continuously overflows over the external surfaces of the patient and onto the operating room floor.[39] In the current model, the mechanically ventilated rat was placed in a box of $CO_2$ to ensure that the relatively shallow abdominal cavity of the rat was exposed to a high concentration of humidified-warm $CO_2$. In both methods, a large portion of the body is bathed in $CO_2$ and therefore both methods are likely to produce similar results. Finally, analysis was only conducted to reveal acute damage to the peritoneum, and longer term consequences such as postoperative adhesion formation were not investigated.

In conclusion, the present study has shown that, in a rat model, exposing the peritoneal mesothelium to conditions that replicate minimum recommended air flow within an operating room causes inadvertent loss of mesothelium that can be prevented by insufflating humidified-warm $CO_2$ into the open abdominal cavity. This finding suggests that humidified-warm $CO_2$ provides a simple method to reduce desiccative damage to the peritoneal mesothelium without the need for intraperitoneal irrigation or wet packs.

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## Disclosure

J.K.M. was as an employee of FPHC during the time the research was conducted and is no longer, but does undertake paid contracted services for FPHC from time to time and owns a small number of shares in FPHC. N.T. and J.vdL have attended research meetings at FPHC, for which expenses were covered by FPHC. J.vdL is a shareholder of Cardia Innovation.

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