2. Malutan AM, Simon I, Ciortea R, Mocan-Hognogi RF, Dudea M, Mihu D. Surgical scar endometriosis: a series of 14 patients and brief review of literature. *Clujul Med*. 2017;90:411–415.
3. Sumathy S, Mangalakanthi J, Purushothaman K, Sharma D, Remadevi C, Sreedhar S. Symptomatology and surgical perspective of scar endometriosis: a case series of 16 women. *J Obstet Gynaecol India*. 2017;67:218–223.
4. Maillot J, Brun JL, Dubuisson V, Bazot M, Grenier N, Cornelis FH. Mid-term outcomes after percutaneous cryoablation of symptomatic abdominal wall endometriosis: comparison with surgery alone in a single institution. *Eur Radiol*. 2017;27:4298–4306.
5. Pas K, Joanna SM, Renata R, Skret A, Barnas E. Prospective study concerning 71 cases of caesarean scar endometriosis (CSE). *J Obstet Gynaecol*. 2017;37:775–778.

https://doi.org/10.1016/j.jmig.2017.12.002

Regarding "Ultrastructural Investigation of Pelvic Peritoneum in Patients with Chronic Pelvic Pain and Subtle Endometriosis in Association with Chromoendoscopy"

Philippe R. Koninckx, MD, PhD[a]
Anastasia Ussia, MD[b]
Leuven, Belgium
Rome, Italy

To the Editor:

We did appreciate the nice pictures in the article on ultrastructural investigation of pelvic peritoneum in women with pain and endometriosis [1]. The use of methylene blue to stain and diagnose endometriosis or damaged peritoneal areas remains debatable since the original observation in 1994 [2].

We are interested to know the time interval between the beginning of laparoscopy and the peritoneal biopsy. Indeed, some of the changes observed could be explained by mesothelial cell trauma by the CO_2 pneumoperitoneum and/or by removing the biopsy specimen through the trocar. Indeed, over the last decade awareness has grown that mesothelial cells are extremely sensitive to any type of trauma and react within seconds by retraction. Exposure to CO_2 pneumoperitoneum thus leads to bulging of cells within 30 minutes of exposure [3]. Unfortunately, this article did not mention that in order to obtain their excellent scanning electron microscopy images in mice, in vivo fixation of the peritoneum had been necessary. Indeed, the time needed to take a peritoneal biopsy specimen by laparoscopy or laparotomy and the unavoidable exposure to CO_2 pneumoperitoneum or, worse, to air with 20% oxygen already induced important mesothelial changes. In addition, saline used for irrigation rapidly damages the mesothelium. This mesothelial reaction [4] and the consequences for postoperative adhesion formation and their prevention by conditioning were recently reviewed [5–7].

Because pathologists generally see more than can be described in an article, this comment is a suggestion to reconsider your results, especially the relationship with endometriosis by taking into account this rather recent observation of rapid mesothelial damage by CO_2 laparoscopy. In addition, we hope to increase awareness of this rapid mesothelial reaction because it is important for future studies on mesothelial morphology.

References

1. Mehdizadehkashi A, Tahermanesh K, Fazel Anvari-Yazdi A, et al. Ultrastructural investigation of pelvic peritoneum in patients with chronic pelvic pain and subtle endometriosis in association with chromoendoscopy. *J Minim Invasive Gynecol*. 2017;24:114–123.
2. Manhes H, Shulman A, Haag T, Canis M, Demontmarin JL. Infertility due to diseased pelvic peritoneum—laparoscopic treatment. *Gynecol Obstet Invest*. 1994;37:191–195.
3. Volz J, Koster S, Spacek Z, Paweletz N. Characteristic alterations of the peritoneum after carbon dioxide pneumoperitoneum. *Surg Endosc*. 1999;13:611–614.
4. Mutsaers SE, Prele CM, Pengelly S, Herrick SE. Mesothelial cells and peritoneal homeostasis. *Fertil Steril*. 2016;106:1018–1024.
5. Koninckx PR, Gomel V, Ussia A, Adamyan L. Role of the peritoneal cavity in the prevention of postoperative adhesions, pain, and fatigue. *Fertil Steril*. 2016;106:998–1010.
6. Koninckx PR, Gomel V. Introduction: quality of pelvic surgery and postoperative adhesions. *Fertil Steril*. 2016;106:991–993.
7. Gomel V, Koninckx PR. Microsurgical principles and postoperative adhesions: lessons from the past. *Fertil Steril*. 2016;106:1025–1031.

https://doi.org/10.1016/j.jmig.2017.10.030

Author's Reply

To the Editor:

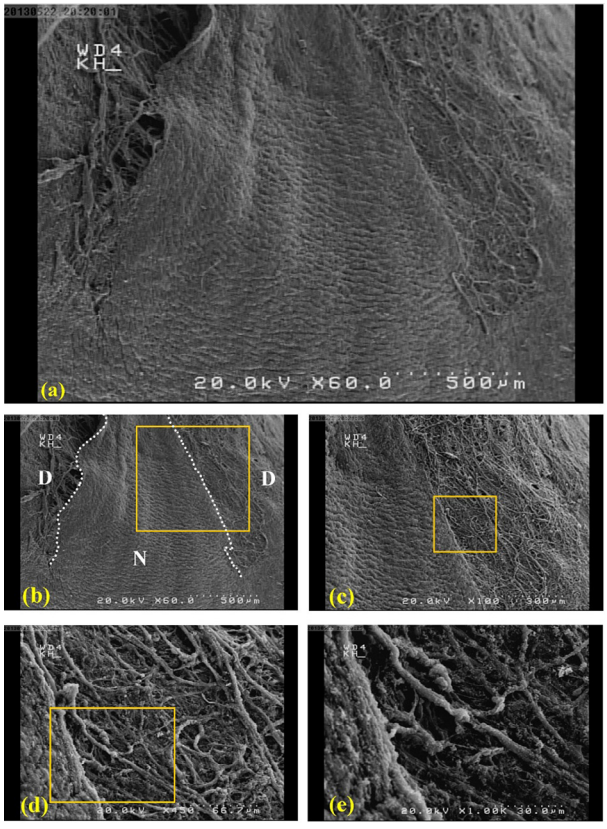
On behalf of all authors of the paper entitled, "Ultrastructural Investigation of Pelvic Peritoneum in Patients with Chronic Pelvic Pain and Subtle Endometriosis in Association with Chromoendoscopy" [1], we thank Drs. Philippe R. Koninckx and Anastasia Ussia for the kind words of appreciation and comments on the scanning electron microscopy (SEM) images. Here we reiterate some comments discussed before in detail by the authors in original article.

In gynecology the idea of staining with methylene blue to identify abnormal peritoneal areas dates back to a 1994 report by Canis et al [2]. As mentioned in the article, most stained areas displayed mild and moderate destruction, and the nano-scale methylene blue granules could settle between the destroyed superficial tangle strands but could not deposit on the denuded cell surfaces (for severe destruction). Hence, all severely destroyed and denuded mesothelial surfaces were colorless. We do agree that the use of methylene blue cannot be a precise diagnostic method for all affected peritoneal areas.

As to the inquiry regarding the time interval, after placement of umbilical and lateral trocars, careful inspection of the pelvic cavity was done in 2 to 3 minutes. Instillation and washing with methylene blue was performed in 5 minutes. To minimize tissue damage, the peritoneum was excised by scissors, grasped, and pulled out gently through an 10 mm Kii Access Systems trocar (Applied Medical, Santa Margarita, CA). This cannula has the advantage of a detachable

Fig

(A) Scanning electron microscopy image of peritoneal tissue damaged by grasping. (B) Dotted lines outline the boundary of normal (*N*) and damaged (*D*) areas. (C–E) Magnification corresponding to the box illustrated [4].



cannula sleeve from the handle during tissue extraction, which allows the excised tissue to be removed without contact and crashing by the trocar silicon CO_2 valve.

To prepare the peritoneal sample for SEM imaging, we immersed the tissues rapidly in 2.5% glutaraldehyde fixation solution at 37°C, washed 3 times with buffer phosphate solution for 30 minutes, and dried chemically with hexamethyldisilazane solution according to our protocol previously described [3]. With many years of experience, our SEM operator team are able to use this drying technique to achieve the best morphologic stability without any structural changes.

In our recently published article [4] we described that tissue grasping can damage the peritoneal surface. The case demonstrated that unintentional ultrastructural changes of the pelvic peritoneum during laparoscopic surgery when grasped by instruments can lead to errors in diagnosis and understanding of the disease process by the pathologists. As shown in Figure, the sharp boundary damage by grasping is obvious and can be noted. Accordingly, inadvertent damage can occur due to laparoscopic instruments and should be taken into consideration during laparoscopic peritoneal tissue dissection for diagnostic and ultrastructural investigations.

In conclusion, tissue damage did not seem to occur because of any physical damage during excision, grasping, and removal from trocar. The total exposure of peritoneal tissue to CO_2 gas was less than 10 to 12 minutes.

Kobra Tahermanesh, MD
Abbas Fazel Anvari-Yazdi, PhD
Tehran, Iran

References

1. Mehdizadehkashi A, Tahermanesh K, Fazel Anvari-Yazdi A, et al. Ultrastructural investigation of pelvic peritoneum in patients with chronic pelvic pain and subtle endometriosis in association with chromoendoscopy. *J Minim Invasive Gynecol*. 2017;24:114–123.
2. Canis M, Manhes H, Mage G. Increased diagnostic accuracy of laparoscopy in endometriosis using indigo carmine: a new technique. *Fertil Steril*. 2011;95:e31, author reply: e32.
3. Mehdizadehkashi A, Tahermanesh K, Chaichian S, et al. How to prepare biological samples and live tissues for scanning electron microscopy (SEM). *Galen Med J*. 2014;3:63–80.
4. Mehdizadehkashi A, Tahermanesh K, Faghih Nasiri B, et al. A case of pelvic peritoneal damage by laparoscopy instruments revealed under scanning electron microscope (SEM). *Iran J Obstet Gynecol Infertil*. 2016;19:28–31.

https://doi.org/10.1016/j.jmig.2017.10.031