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Trimmed central venous catheters do not increase endothelial injury in an ovine model

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Abstract

Introduction: Central venous catheters (CVCs) are often trimmed during heart transplantation and pediatric cardiac surgery. However, the risk of endothelial injury caused by the cut tip of the CVC has not been evaluated. We hypothesized that there is no difference in the degree of endothelial injury associated with trimmed CVCs versus standard untrimmed CVCs.

Methods: In four adult male sheep, the left external jugular vein was exposed in three segments, one designated for an untouched control group, one for the trimmed CVC group, and one for the untrimmed CVC group. Trimmed and untrimmed CVC tips were rotated circumferentially within their respective segments to abrade the lumen of the vein. The vein samples were explanted, and two representative sections from each sample were analyzed using hematoxylin and eosin (H&E) staining, as well as with immunohistochemistry against CD31, von Willebrand factor (vWF), endothelial nitric oxide synthase (eNOS), and caveolin. Higher immunohistochemical stain distributions and intensities are associated with normal health and function of the venous endothelium. Data are presented as counts with percentages or as means with standard error.

Results: H&E staining revealed no evidence of endothelial injury in 6/8 (75%) samples from the untouched control group, and no injury in 4/8 (50%) samples from both the trimmed and untrimmed CVC groups ($p = 0.504$). In all remaining samples from each group, only mild endothelial injury was observed. Immunohistochemical analysis comparing trimmed CVCs versus untrimmed CVCs revealed no difference in the percentage of endothelial cells staining positive for CD31 ($57.5\% \pm 7.2\%$ vs $55.0\% \pm 9.2\%$, $p = 0.982$), vWF ($73.8\% \pm 8.0\%$ vs $62.5\% \pm 9.6\%$, $p = 0.579$), eNOS ($66.3\% \pm 4.2\%$ vs $63.8\% \pm 7.5\%$, $p = 0.962$), and caveolin ($53.8\% \pm 5.0\%$ vs $51.3\% \pm 4.4\%$, $p = 0.922$). There were no significant differences between the groups in the distributions of stain intensity for CD31, vWF, eNOS, and caveolin.

Conclusion: Trimmed CVCs do not increase endothelial injury compared to standard untrimmed CVCs.

Keywords

Catheter, cardiac surgery, trim, endothelium, injury

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Introduction

Central venous catheters (CVC) are essential tools in the operative and critical care settings, allowing for the rapid infusion of resuscitative fluids and the direct administration of medications into the central venous system. More than 5 million CVCs are used each year in the United States, with preference for insertion in the internal jugular vein to reduce the risk of catheter-related bloodstream infections.[1] To further minimize infection

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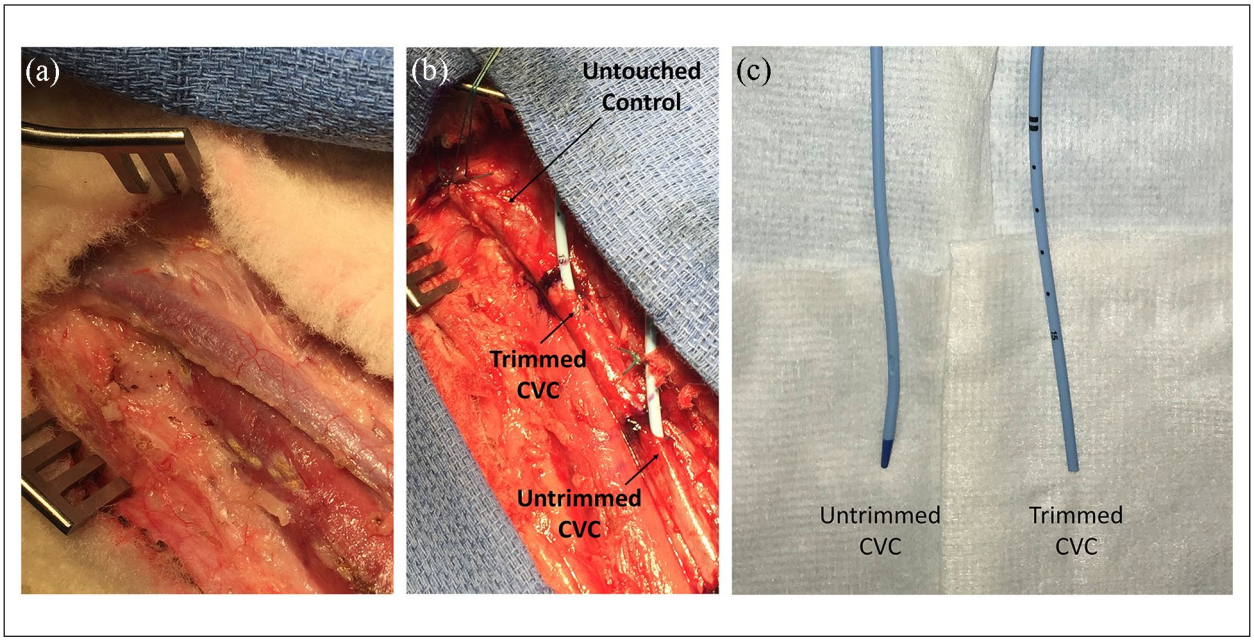

Figure 1. (a) The left external jugular vein of the sheep is exposed, (b) distinct segments are designated for the untouched control group, the trimmed central venous catheter (CVC) group, and the untrimmed CVC group, and (c) the tips of the trimmed and untrimmed CVCs are shown.

risk, excessive CVC manipulation or repositioning is avoided after initial placement.[2] As a result, situations may arise during cardiac surgery in which an internal jugular CVC may be intentionally trimmed at its tip in the operative field. During heart transplantation using the bicaval technique, for example, internal jugular CVCs are often cut when the recipient superior vena cava is divided and the native heart is explanted. In addition, during pediatric cardiac surgery involving the right atrium or superior vena cava, CVCs are often cut shorter while on cardiopulmonary bypass to optimize catheter positioning and avoid the risks of having the CVC crossing a cavoatrial anastomosis or residing in the right atrium.[3,4] In these situations, the trimmed CVC is maintained and continues to function in the postoperative setting, reducing the number of procedures needed and eliminating the additional risks associated with CVC reinsertion and repositioning.

While prior clinical studies have demonstrated that trimmed CVCs can be safely and effectively utilized after cardiac surgery without significant risk of infection or thrombus formation,[3] an additional concern that has not been previously investigated is the risk of endothelial injury due to abrasion of the venous wall by the cut tip of the CVC. Here, we used an in vivo large animal model to histologically examine whether trimmed CVCs may induce excess endothelial damage to the jugular vein. We hypothesized that there would be no difference in the degree of endothelial injury associated with trimmed CVCs versus standard untrimmed CVCs.

Methods

Animal care and use

All experiments were performed in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals 8th Edition and approved by the Institutional Animal Care and Use Committee at Stanford University (Protocol 28943). This serves as our ethics statement. Because no human subjects were involved, informed consent was not applicable.

Endothelial injury model

Four adult male 50 kg Dorset sheep were used for this study. Briefly, these sheep were concurrent subjects in an unrelated study and had previously undergone left thoracotomy and ascending aortotomy on cardiopulmonary bypass to assess the effect of various adhesion barriers in an ovine model of cardiac surgery.[5] Each sheep was recovered and planned for terminal assessment of intrathoracic adhesion formation 1 month later, at which time the present study was concomitantly performed.

For the terminal surgery, each sheep was sedated with intravenous diazepam (0.2 mg/kg) and endotracheally intubated. Anesthesia was maintained with inhaled isoflurane (1.5%–3%). The sheep were positioned in right lateral decubitus position and a longitudinal incision was made over the left neck to expose the external jugular vein (Figure 1(a)), which serves as the dominant venous drainage for the ipsilateral head in sheep. The left external

jugular vein, which had not been manipulated in any way prior to the present study, was partitioned into three 3 cm segments, one designated for an untouched control group, one for the trimmed CVC group, and one for the untrimmed CVC group (Figure 1(b)). An Arrowg+ard Blue PLUS 7 Fr triple-lumen CVC (Teleflex Inc., Wayne, PA, USA) was cut using Metzenbaum scissors to produce the trimmed and untrimmed CVC tips (Figure 1(c)). In the trimmed CVC segment, the external jugular vein was accessed using an 18 Ga introducer needle and the trimmed CVC was advanced into the vein using Seldinger technique, keeping within the designated segment. The same process was repeated for the untrimmed CVC segment using the untrimmed CVC tip. The segment for the untouched control group was left unmanipulated. Finally, the trimmed and untrimmed CVC tips were rotated circumferentially within and along the length of their respective segments for 10 total rotations, thereby abrading the internal lumen of the vein. The sheep were then euthanized by potassium chloride injection to assess intrathoracic adhesion formation, as previously described.[5] The left external jugular vein was explanted for histological analysis.

Histological preparation and analysis

Each vein sample was filled and submerged in optimum cutting temperature compound (Thermo Fisher Scientific, Waltham, MA, USA), frozen using 2-methylbutane on dry ice, and stored at −80°C. Short-axis sections with 10 µm thickness through each vein sample were prepared and placed on SuperFrost microscope slides (Thermo Fisher Scientific). Two slides were selected for each vein sample, one representing the proximal portion of the sample and one representing the distal portion of the sample.

Hematoxylin and eosin (H&E) staining was performed using the Thermo Scientific Shandon Rapid-Chrome H&E Frozen Section Staining Kit (Thermo Fisher Scientific), and 20× images of the vessel lumen were obtained using a Keyence BZ-X810 microscope and BZ-X88 Analyzer software (Keyence, Osaka, Japan). Immunohistochemical staining was performed by HistoWiz Inc. (Brooklyn, NY, USA) following their standard operating procedure, using a BOND RX autostainer (Leica Biosystems, Wetzlar, Germany) with enzyme treatment (1:1000). BOND Polymer Refine Detection (Leica Biosystems) was used according to the manufacturer's protocol. After staining, sections were dehydrated and film coverslipped using a TissueTek-Prisma and Coverslipper (Sakura, Torrance, CA, USA). Whole slide scanning (40×) was performed on an Aperio AT2 (Leica Biosystems). Samples were stained with primary antibodies against CD31 (Abcam, Cambridge, UK, ab28364, 1:100), von Willebrand factor (vWF, Abcam, ab6994, 1:8000), endothelial nitric oxide synthase (eNOS, Abcam, ab5589, 1:200), and caveolin (Abcam, ab87770, 1:150), for which higher levels of stain distribution and intensity are positively associated with normal health and function of the venous endothelium.[6,7] Secondary antibodies were goat anti-rabbit IgG antibody (Vector Laboratories, Newark, CA, USA, AI-1000-1.5, 1:100) for CD31, vWF, and eNOS, and donkey anti-goat IgG antibody (Abcam, ab205723, 1:100) for caveolin.

Histological analysis, including the extent of endothelial injury by H&E staining, the percentage of endothelial cells positive for each immunohistochemistry stain, and the degree of immunohistochemistry stain intensity, was performed by a clinically trained pathologist in a fully blinded fashion.

Statistical analysis

Statistical analyses were performed using Stata version 14.2 (StataCorp LLC., College Station, TX, USA). Continuous variables were reported as mean ± standard error and compared using one-way analysis of variance, with Tukey's test for pairwise comparisons. Categorical variables were reported as counts with percentages and compared using chi-square tests, with Fisher's exact test for pairwise comparisons. For all comparisons, p-value <0.05 was considered statistically significant. Data will be made available upon reasonable request.

Results

Upon gross inspection, there was no evidence of injury to the endothelium for any sample. Microscopically, H&E staining revealed no evidence of endothelial injury in 6/8 (75%) samples from the untouched control group, and no injury in 4/8 (50%) samples from both the trimmed and untrimmed CVC groups ($p=0.504$, Figure 2, Supplemental Table 1). In all remaining samples from each group, only mild endothelial injury was observed. No samples in any group had moderate or severe endothelial injury on H&E analysis.

Immunohistochemistry analysis comparing trimmed CVCs versus untrimmed CVCs revealed no difference in the percentage of endothelial cells staining positive for CD31 (57.5% ± 7.2% vs 55.0% ± 9.2%, $p=0.982$, Figure 3(a)–(d)), vWF (73.8% ± 8.0% vs 62.5% ± 9.6%, $p=0.579$, Figure 4(a)–(d)), eNOS (66.3% ± 4.2% vs 63.8% ± 7.5%, $p=0.962$, Figure 5(a)–(d)), and caveolin (53.8% ± 5.0% vs 51.3% ± 4.4%, $p=0.922$, Figure 6(a)–(d)). The untouched control samples generally exhibited a higher percentage of positive-stained cells compared to both the trimmed and untrimmed CVCs (CD31 61.3% ± 11.1%, $p=0.954$ vs trimmed, $p=0.894$ vs untrimmed; vWF 78.8% ± 5.5%, $p=0.895$ vs trimmed, $p=0.330$ vs untrimmed; eNOS 82.5% ± 7.7%, $p=0.222$ vs trimmed, $p=0.142$ vs untrimmed), although this difference was only statistically significant for caveolin (75.0% ± 5.0%, $p=0.018$ vs trimmed, $p=0.009$ vs untrimmed).

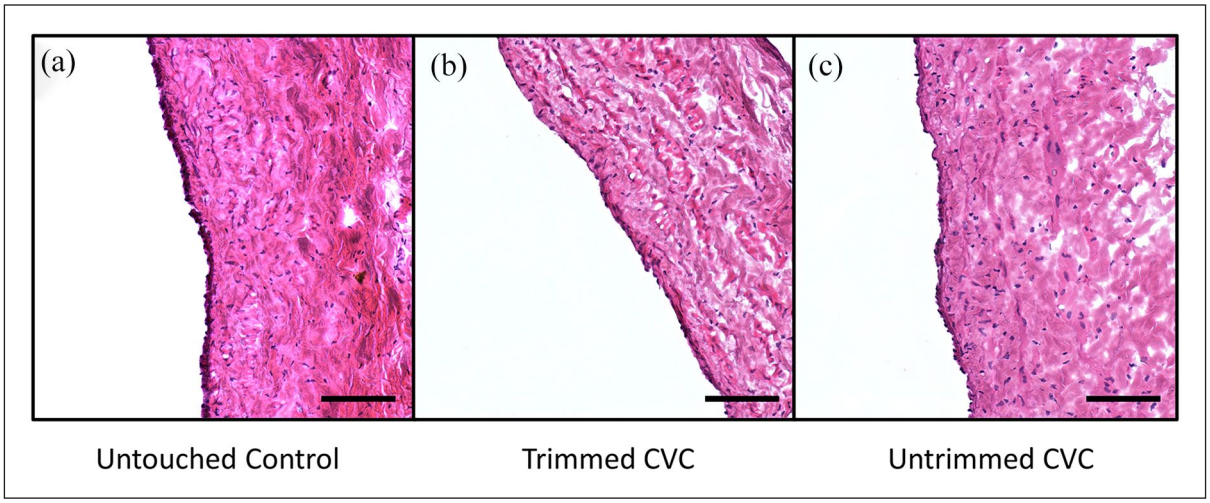

Figure 2. Vein sections stained with hematoxylin and eosin are visualized under brightfield microscopy for (a) the untouched control group, (b) the trimmed central venous catheter (CVC) group, and (c) the untrimmed CVC group. Scale bar represents 100 µm.

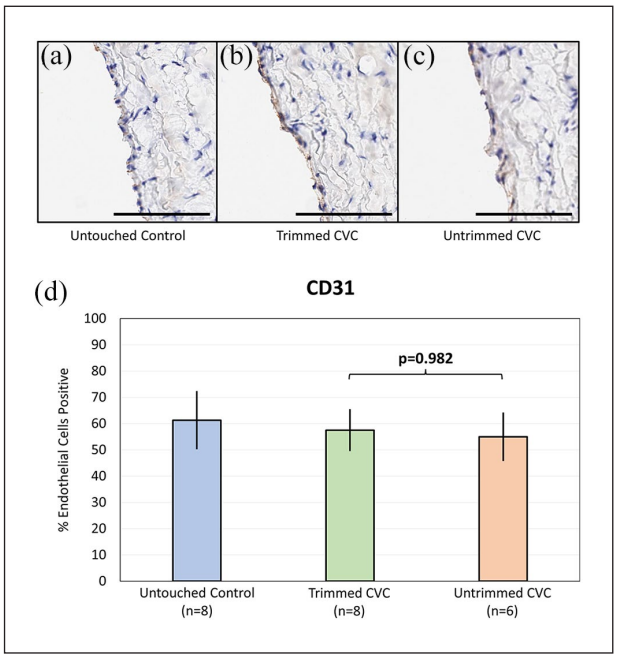

Figure 3. Immunohistochemistry against CD31 was performed for vein sections from (a) the untouched control group, (b) the trimmed central venous catheter (CVC) group, (c) the untrimmed CVC group. Scale bar represents 100 µm, and (d) the mean percentage of positively stained endothelial cells in each group is shown, with error bars representing standard error.

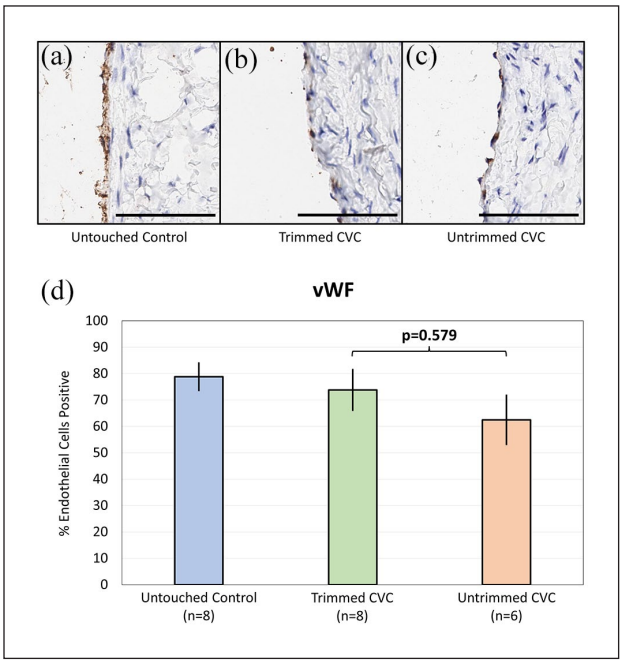

Figure 4. Immunohistochemistry against von Willebrand factor (vWF) was performed for vein sections from (a) the untouched control group, (b) the trimmed central venous catheter (CVC) group, (c) the untrimmed CVC group. Scale bar represents 100 µm, and (d) the mean percentage of positively stained endothelial cells in each group is shown, with error bars representing standard error.

Immunohistochemistry analysis comparing trimmed CVCs versus untrimmed CVCs further revealed no significant difference in the distributions of stain intensity (Supplemental Table 2). For CD31, all 8/8 (100%) of trimmed CVC samples had 1+ intensity compared to 5/6 (83.3%) of untrimmed CVC samples ($p=0.429$), whereas 6/8 (75.0%) of untouched control samples had 1+ intensity (overall $p=0.167$). For vWF, 7/8 (87.5%) of trimmed CVC samples had 1+ or 2+ intensity compared to 6/8 (75.0%) of untrimmed CVC samples ($p=0.354$), whereas 5/8 (62.5%) of untouched control samples had 1+ or 2+ intensity (overall $p=0.280$). For eNOS, 5/8 (62.5%) of trimmed CVC samples had 3+ intensity compared to 6/8 (75.0%) of untrimmed CVC

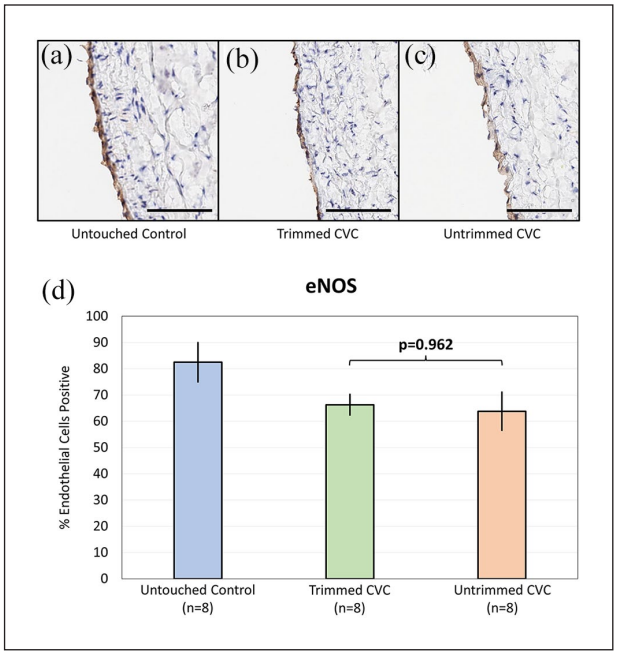

Figure 5. Immunohistochemistry against endothelial nitric oxide synthase (eNOS) was performed for vein sections from (a) the untouched control group, (b) the trimmed central venous catheter (CVC) group, (c) the untrimmed CVC group. Scale bar represents 100 µm, and (d) the mean percentage of positively stained endothelial cells in each group is shown, with error bars representing standard error.

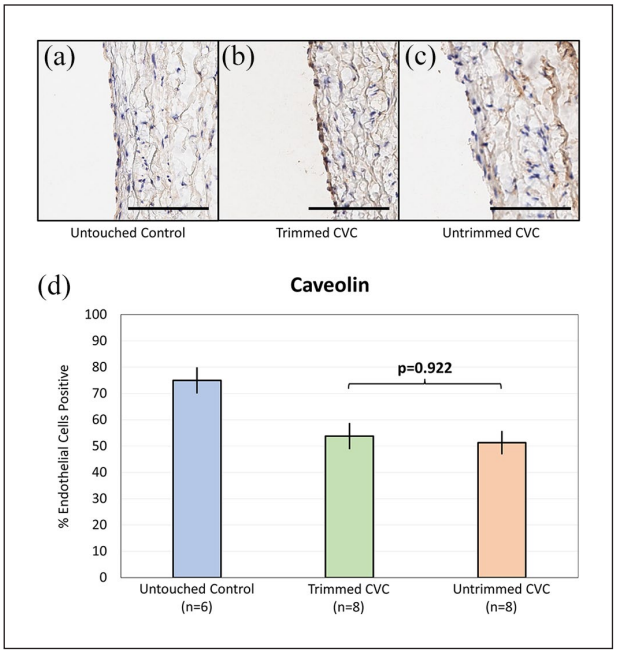

Figure 6. Immunohistochemistry against caveolin was performed for vein sections from (a) the untouched control group, (b) the trimmed central venous catheter (CVC) group, (c) the untrimmed CVC group. Scale bar represents 100 µm, and (d) the mean percentage of positively stained endothelial cells in each group is shown, with error bars representing standard error.

samples ($p > 0.999$), whereas all 8/8 (100%) of untouched control samples had 3+ intensity (overall $p = 0.171$). Finally, for caveolin, all 8/8 (100%) of trimmed CVC samples had 1+ or 2+ intensity compared to 7/8 (87.5%) of untrimmed CVC samples ($p = 0.467$), whereas all 6/6 (100%) of untouched control samples had 1+ or 2+ intensity (overall $p = 0.063$).

Discussion

Trimmed peripherally inserted central catheters have previously been linked with a potential increased risk of infection and thrombus formation.[8,9] Borrowing upon these observations in peripherally inserted central catheters, similar concerns have been raised for trimmed CVCs in the internal jugular position. Indeed, previous studies have shown that even intact catheter tips can cause mechanical injury to the venous endothelium due to repetitive motion against the wall, leading over time to thrombus formation and a proliferative effect within the venous wall similar to intimal hyperplasia.[10] Whether trimmed CVCs may induce greater endothelial damage compared to untrimmed intact CVCs, however, remains unknown.

Recently, Glenski et al.[3] studied trimmed CVCs in pediatric cardiac surgery patients at their medical center between 2018 and 2020, and 147 (35%) of their 420 patients had trimmed CVCs during and after surgery. The CVCs were intentionally cut because catheters extending too far into the atrium may present technical challenges during surgery, and increase the risk of atrial perforation or thrombosis, tachyarrhythmias, valvular incompetence, and catheter dysfunction.[11] Leaving a CVC across a cavoatrial anastomosis may also lead to chronic scar formation and increase the risk of anastomotic stenosis.[4] If the catheters were partially withdrawn from the skin instead of trimmed internally, however, the exposed external portion may increase the risk of central line-associated blood stream infections.[12] In their study, Glenski et al.[3] observed zero cases of infection, thrombus formation, or catheter occlusion, suggesting that trimmed CVCs may be safe in this clinical scenario after cardiac surgery.

While Glenski et al.[3] evaluated the concern for infection, thrombus formation, and catheter occlusion after trimming CVCs, thus far it remains unknown whether the cut tip of trimmed CVCs may cause injury to the venous endothelium on a microscopic level. Here, we investigated the concern for endothelial injury using a combination of light microscopy and immunohistochemistry techniques. To create a large animal model for venous endothelial injury, we applied rotational and longitudinal motion to trimmed and untrimmed CVCs within the external jugular vein of sheep. This manipulation mimics the potential

abrasive motion of CVCs within the jugular vein lumen in human patients, although the degree of manipulation applied to each catheter in this experimental setting was far more than what would be expected in the clinical setting, thus magnifying the likelihood and extent of injury.

To detect evidence of injury, we employed standard light microscopy analyses of H&E-stained cross-sections of veins from the untouched control group, and from the trimmed CVC and untrimmed CVC groups. We additionally performed immunohistochemistry analyses using several well-established markers for venous endothelial health and function, based on the methodology utilized by prior studies examining saphenous vein endothelial integrity after open versus endoscopic harvesting.[6,7,13] Specifically, we selected CD31, vWF, eNOS, and caveolin. CD31, also known as platelet/endothelial cell adhesion molecule-1, is highly expressed by endothelial cells, especially at cell-cell junctions, and plays an essential role in maintaining and restoring the integrity of endothelial cell linings after injuries to the vascular permeability barrier.[14] vWF, a glycoprotein required for normal hemostasis, is synthesized by endothelial cells, and both circulating levels and venous endothelial levels have been used as markers for vascular injury.[7,15] Caveolin is a membrane protein involved in numerous essential signaling pathways for maintaining endothelial cell function and homeostasis, including the production of eNOS which is responsible for generating nitric oxide.[16] Decreased and displaced expression of eNOS and caveolin may signify endothelial damage.[7]

As expected, the vein samples in the untouched control group exhibited high percentages of endothelial cells expressing CD31, vWF, eNOS, and caveolin, indicating minimal injury in this negative control group. H&E analysis of the vein samples in the untouched control group also demonstrated predominantly no endothelial injury. Both the trimmed and untrimmed CVC groups, however, exhibited lower percentages of endothelial cells expressing CD31, vWF, eNOS, and caveolin than the untouched control group, suggesting that our injury model was successful. Importantly, we observed a strong concordance between the trimmed and untrimmed CVC groups in the percentage of endothelial cells expressing CD31, vWF, eNOS, and caveolin, as well as in the distribution of stain intensity for these markers. Overall, these findings support our hypothesis that there is no difference in the degree of endothelial injury associated with trimmed CVCs versus standard untrimmed CVCs.

We acknowledge several important limitations of this study. First, our sample size was small, and the sheep utilized for this study were concurrent subjects in another experiment. However, given that the other experiment was isolated to the thoracic cavity and the left external jugular vein of these sheep had not been previously manipulated,[5] we do not expect the results of the present study to be significantly affected. Each sheep also served as its own control in the present study, which further reduces the potential for bias due to inter-subject differences. It is important to note, however, that our study was designed to specifically examine endothelial injury in the acute setting due to excessive catheter manipulation, whereas in the clinical arena, CVCs remain within the venous lumen for days or longer and may cause damage over a longer period of time. Such chronic injury to vessels may be evidenced by proliferative effects, including fibrotic changes, stenosis, or occlusion, which we were unable to observe with our current experiments. In the future, chronic injury studies may be performed to assess the impact of retaining trimmed versus untrimmed CVCs. Lastly, we selected multiple markers of endothelial health to assess damage to the endothelial lining, informed by other experiments studying saphenous veins.[6,7,13] However, it is possible that light microscopy and immunohistochemistry may not provide a complete assessment, and that other techniques are needed to detect a difference in endothelial injury. For example, electron microscopy of the luminal surface of the vein may be useful to fully understand the nature of endothelial changes induced by CVCs.[9,17] Moreover, analyzing the biomechanical roughness of the catheter tips after trimming may also be an interesting topic for future research, especially as catheters cut sharply with a scalpel and catheters cut roughly with scissors will have different biomechanical roughness and potentially cause different degrees of endothelial injury.[9,17]

Overall, we did not find evidence that trimmed CVCs increase endothelial injury compared to standard untrimmed CVCs. In combination with prior work showing that trimmed CVCs have a minimal risk of causing infection, thrombus formation, or catheter occlusion, our results suggest that trimmed CVCs may be safe to use in the postoperative setting, avoiding the risks of infection and endothelial injury associated with manipulating or reinserting an existing CVC.

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Declaration of conflicting interests

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Supplemental material

Supplemental material for this article is available online.

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