Histologic changes associated with the use of fibrinogen- and thrombin-impregnated collagen in the prevention of pulmonary air leakage

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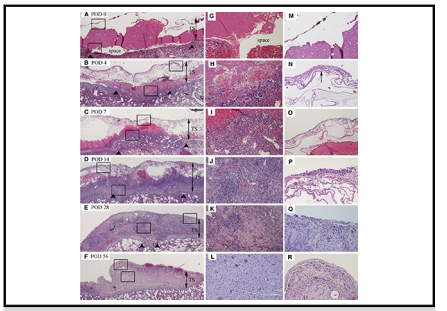
ABSTRACT

Objective: Although fibrinogen- and thrombin-impregnated collagen (TachoSil; Takeda GmbH, Linz, Austria) can be applied to prevent air leakage, the impact of its use on lung healing is unknown. Therefore, we histologically evaluated the long-term healing process associated with the use of TachoSil to prevent air leakage in a canine model.

Methods: Via left thoracotomy, visceral pleural defects of 10 × 10 mm were created on each lung lobe of female beagles. After air leakage was confirmed, each pleural defect was covered with TachoSil. The repair sites were histologically evaluated on postoperative days 0, 4, 7, 14, 28, and 56.

Results: All animals survived, and none developed pneumothorax. Histologically, inflammatory cells infiltrated the TachoSil from the pleural defect, and pleural mesothelium comprised the regenerated outermost layer of the TachoSil soon after the surgery. Inflammatory cells, myofibroblasts, and neovascular vessels subsequently spread over the entire TachoSil. The number of inflammatory cells decreased, and myofibroblast and neovascular vessels replaced the entire TachoSil. In addition, the elastic layer started to regenerate from both edges and completely repaired the pleural defect. The lung parenchyma around the pleural defects was not influenced throughout the observational period, because these healing processes occurred only inside the TachoSil.

Conclusions: TachoSil provided a mechanical scaffold on which healing could proceed, followed by biodegradation over the long term. TachoSil safely repaired the pleural defects without affecting lung parenchyma. (J Thorac Cardiovasc Surg 2015;149:982-8)



The healing processes that air leakage was prevented with TachoSil.

Central Message

TachoSil safely prevents air leakage without affecting lung parenchyma. It provided a mechanical scaffold on which healing could proceed, followed by biodegradation over the long term.

Perspective

Although TachoSil can be applied to prevent air leakage, the impact of its use on lung healing is unknown. This study histologically showed the long-term healing processes associated with TachoSil, which is used to prevent air leakage. TachoSil provided a mechanical scaffold on which healing could proceed, with subsequent replacement by myofibroblasts and neovascular vessels. TachoSil safely prevented air leakage without negatively affecting the lung parenchyma and without leaving remaining foreign material.

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Postoperative air leakage is a frequent complication of pulmonary resection.[1,2] Persistent air leakage has negative consequences on morbidity, culminating in a lengthy hospitalization with negative economic effects and delayed adjuvant treatment.[3-7] Therefore, a safe and effective procedure is required to prevent and control air leakage.

TachoSil (Takeda GmbH, Linz, Austria) has been successfully used to reduce the prevalence of the complication of air leakage after pulmonary resection.[2,3,8-11] However, the impact of TachoSil use on lung healing is unknown. Therefore, we histologically evaluated the long-term healing processes associated with the use of TachoSil to prevent air leakage in a canine model.

MATERIALS AND METHODS

Animals and Initial Operation

Female beagles (Kitayama Labes Co Ltd, Nagano, Japan) were preanesthetized with ketamine (10 mg/kg) and atropine sulfate (0.5 mg), intubated under general anesthesia (5 mg/kg propofol intravenously; 1 mg/kg succinylcholine intravenously), and placed under controlled ventilation. Cefazolin (1 g) was intraoperatively administered. Anesthesia was maintained by periodic injections of propofol and succinylcholine.

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Abbreviations and Acronyms
α-SMA = α-smooth muscle actin
POD = postoperative day

Surgical Procedure

Animals were placed in the right lateral position for a left thoracotomy. Visceral pleural defects of 10 × 10 mm were created on the side of the chest wall, the mediastxinal side, and the interlobar side by sharp dissection. We dissected lung parenchyma to the depths confirming bleeding and air leakage from the created pleural defect. Constriction was applied if needed, but cautery was not. TachoSil was applied to cover each pleural defect with an overlap of more than 5 mm and held against the lung tissue for 5 minutes (Figure 1). We macroscopically confirmed that TachoSil firmly attached to the defect. No visible air leakage was confirmed. The chest wall was closed after air evacuation by chest tube. After no air leakage was observed, the chest tube was removed before the dogs were extubated. The animals were returned to their cages after being injected intramuscularly with ketoprofen (1 mg/kg).

Animals were euthanized by a pentobarbital overdose on postoperative days (PODs) 0, 4, 7, 14, 28, and 56. Six dogs were used; 1 was killed on each POD. Three or 4 pleural defects were created in 1 dog for each time point. Thoracotomy in a different intercostal space was performed to macroscopically evaluate postoperative pleural adhesions. The left lung and the surrounding adherent tissue were harvested for each lobe except for the case processed on POD 14, in which en bloc resection was performed because of interlobar adhesion.

Histologic Findings

Specimens were fixed in 10% buffered formalin, and the pleura covered by TachoSil was serially cut into 5 μm–thick slices. Because TachoSil was not detected by macroscopic findings on POD 56, the entire lobe was serially cut into 5 μm–thick sections and then all paraffin-embedded sections were evaluated by hematoxylin–eosin staining. Pleural defects and the proliferation of myofibroblasts and neovascular vessels were respectively assessed by staining the sections with Elastica van Gieson, α-smooth muscle actin (α-SMA), and CD31. The expression levels of each cell were defined as follows: 1+ = mean low levels, 2+ = intermediate levels, and 3+ = high levels of inflammatory cells, myofibroblasts, and neovascular vessels. The Ethics Committee of Hiroshima University approved the study protocol, and laboratory animal care proceeded according to the guidelines for laboratory animal care.[12]

RESULTS

Macroscopic Findings

A total of 20 TachoSil patches (3-4 per dog) were applied to pleural defects in the dogs. All dogs survived without severe complications throughout the observation period. Further, all TachoSil patches were attached to the lung parenchymal surface. Extensive inflammation or pneumothorax was not observed at the time of sacrifice (Table 1). Two adhesions (10%) were found in the pleura covered by TachoSil: 1 each in the interlobar and mediastinal pleura on PODs 7 and 14, respectively. TachoSil was macroscopically detectable until POD 28 and only microscopically detectable on POD 56.

Microscopic Findings

Pleural defects were confirmed as spaces caused by air leakage on POD 0 (Figure 2, *A*) and as the loss of the elastic layer on Elastica van Gieson–stained sections (Figure 3). The time-dependent changes of each of the cells are summarized in Table 1. The mesothelium had formed and covered the outermost layer of the TachoSil after POD 4 (Figure 2, *N*). Inflammatory cells such as lymphocytes and neutrophils initially infiltrated the space, which became filled with these cells and blood cells on POD 4 (Figure 2, *B*). Subsequently, inflammatory cells spread widely to the TachoSil from POD 7 (Figure 2, *C*) to 28 (Figure 2, *E*), but the cell numbers had decreased by POD 56 (Figure 2, *F*). However, the myofibroblasts and neovascular vessels exhibited histologic characteristics that differed from those of the inflammatory cells. The myofibroblasts and neovascular vessels formed in the TachoSil around the pleural defects on POD 7 (Figure 4, *C* and *I*), which was after the inflammatory cells were first observed, and increased progressively from POD 7 to 28 (Figure 4, *E* and *K*). Finally, the myofibroblasts and neovascular vessels remained in the TachoSil, occupying the entire TachoSil on POD 56 (Figure 4, *F* and *L*). In addition, by POD 14, the pleural defects were confirmed to be loss of the elastic layer (Figure 3, *A-D*). However, elastic layer regeneration originated from both edges, and pleural defects started to decrease on POD 28 (Figure 3, *E*). The pleural defect was completely repaired by POD 56 (Figure 3, *F*). Because these healing processes occurred only inside the TachoSil, the lung parenchyma around the pleural defects was not influenced throughout the observational period.

DISCUSSION

This study histologically demonstrated the long-term healing processes associated with TachoSil, which is used to prevent air leakage. TachoSil safely repaired pleural defects to prevent air leaks without negatively affecting the lung parenchyma and without leaving remaining foreign material. TachoSil provided a mechanical scaffold on which healing could proceed, followed by biodegradation over the long term.

Because 90% of air leaks resolve by POD 7,[13] we included 2 observation end points in this study: prevention of air leakage in the early postoperative period (PODs 0, 4, and 7) and assessment of possible adverse effects in the late period (PODs 14, 28, and 56). The pleural spaces responsible for air leakage were replaced with inflammatory cells and blood cells on POD 4, and these changes became pronounced on POD 7. In addition, the pleural area around the defects adhered to the TachoSil, and these histologic changes were responsible for prevention of air leakage. A previous study[14] demonstrated the histologic changes associated with the use of fibrin glue-coated collagen fleece to repair pleural defects until POD 7, and these findings were consistent with our results. However, the changes in the TachoSil and their long-term effects on the lung parenchyma have remained unclear. The present study showed

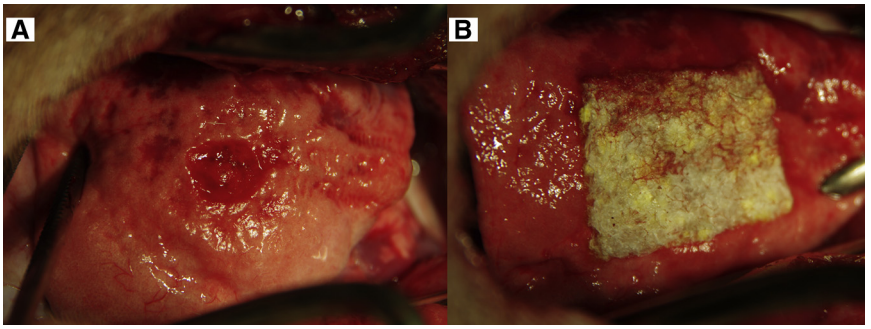

FIGURE 1. Pleural defects and TachoSil (Takeda GmbH, Linz, Austria) cover. Pleural defects of 10 × 10 mm were created by sharp dissection (A). TachoSil covers pleural defect with overlap greater than 5 mm (B). TachoSil collagen patch coated with human fibrinogen and thrombin.

that the TachoSil was completely biodegraded over the long term without negatively affecting the lung parenchyma. After inflammatory cells infiltrated the pleural defects, myofibroblasts and neovascular vessels formed and spread over the entire TachoSil with the inflammatory cells. Finally, the number of inflammatory cells decreased, and the myofibroblasts and neovascular vessels replaced the TachoSil. The TachoSil did not remain as a foreign material. Adverse effects on the lung parenchyma were not found because these healing processes occurred only inside the TachoSil.

In addition, few adhesions were detected in the area covered by the TachoSil, and several possibilities can be proposed to explain this finding. First, although an inflammatory reaction occurred within the TachoSil, it did not occur beyond the TachoSil. Second, the outermost layer of the TachoSil became covered with 1 layer of mesothelium from POD 4, and a rapidly developing mesothelium could prevent adhesions. A relationship between TachoSil and adhesions has been identified in intra-abdominal,[15] pericardial,[16] and gynecologic[17] regions. To our knowledge, pleural adhesions have been prevented only in a few studies in rat models.[18,19] One study in a rat model[19] showed that the pleural defects that were healed by the use of hemostatic fleece became covered by regular mesothelium, and the fleece prevented the development of pleural adhesions. Further, hemostatic fleece promoted rapid mesothelial recovery and reduced plasminogen activator inhibitor-1 levels, leading to the biochemical inhibition of adhesion formation.[18]

The myofibroblasts and elastic layer comprised an important part of the healing process. Myofibroblasts regulate the remodeling of connective tissue by combining the extracellular matrix-synthesizing features of fibroblasts with the cytoskeletal characteristics of contractile smooth muscle cells.[20] Myofibroblasts that are characterized by the expression of α-SMA represent key players in the physiologic reconstruction of connective tissue after injury and in generating the pathologic tissue deformations that characterize fibrosis.[20-24] Our findings demonstrate that similar processes occurred during the healing of pleural defects by TachoSil because the α-SMA–positive layer, which indicates the presence of myofibroblasts, gradually thickened from POD 7 to 56 inside the TachoSil. Excessive and persistent myofibroblast activities transform beneficial tissue repair into the detrimental tissue deformities characteristic of organ fibrosis.[20] However, the myofibroblasts acted only inside the TachoSil, and not in the lung parenchyma, without showing evidence of excessive activity. The elastic layer was also involved in the healing procedure. This study demonstrated that the elastic layer regenerated from both edges on POD 28, with the pleural defects disappearing by POD 56. We previously reported that the processes associated with the TachoComb (Takeda GmbH) during healing of an injured pulmonary artery

TABLE 1. Surgical and postoperative pathologic findings of TachoSil (Takeda GmbH, Linz, Austria)

POD	0	4	7	14	28	56
No. of TachoSil patches for 1 dog	n = 3	n = 4	n = 3	n = 4	n = 3	n = 3
Adhesion	0	0	1	1	0	0
Macroscopic confirmation	+	+	+	+	+	−
Regeneration of mesothelium	−	+	+	+	+	+
Inflammatory cells	−	+	3+	3+	2+	−
Myofibroblasts	−	+	+	2+	3+	3+
Neovascular vessels	−	+	2+	2+	2+	2+
Regeneration of elastic layers	−	−	−	−	+	+

POD, Postoperative day.

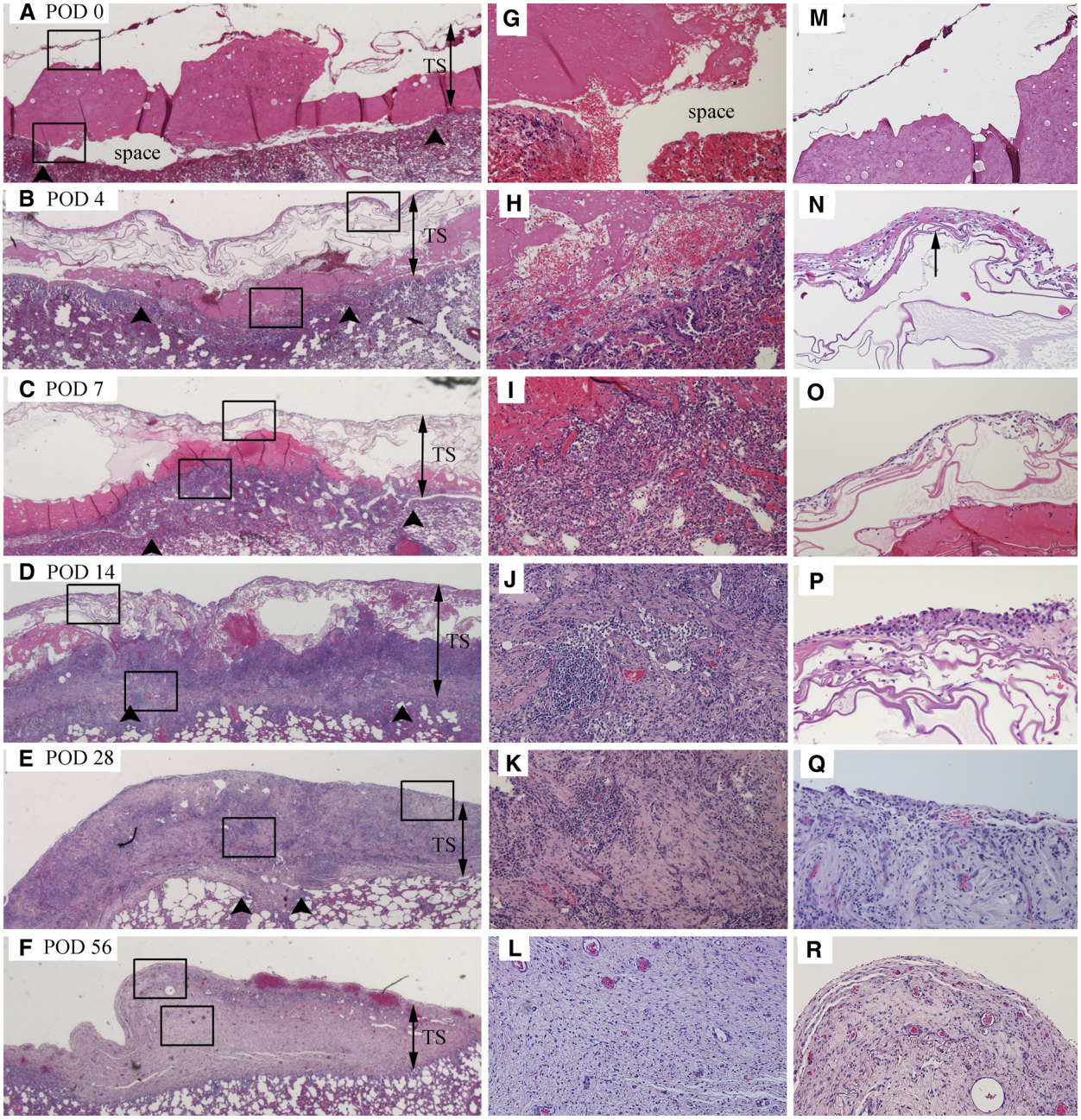

FIGURE 2. Postoperative hematoxylin–eosin staining. Postoperative hematoxylin–eosin staining visualized using loupe (A-F). Around pleural defects (G-L), magnification, ×100. Outermost layer of TachoSil (M-R), magnification, ×100. *Arrowheads* shows both edges of elastic layer (confirmed by Elastica van Gieson staining in Figure 3). Inflammatory infiltration was noted immediately on POD 4, increased progressively from POD 4 to 28, and was resolved by POD 56. In addition, mesothelium (*arrow*) covers the outermost layer of TachoSil on POD 4 (N). TachoSil collagen patch coated with human fibrinogen and thrombin. *POD*, Postoperative day; *TS*, TachoSil.

started to extend into the defect between the endothelial layer and the TachoComb from both edges.[25] The present findings were similar, although the reason for regeneration of the elastic layer remains unknown.

Study Limitations

First, the canine model differed from the condition that is observed in a clinical setting. Clinically, postoperative air leakage often occurs in patients with emphysematous or fibrotic patterns, whereas the lung was normal in the canine model used in this study. In addition, the pleural defects were small and created without removal of the lung. Thus, the pleural defects may have healed relatively quickly with complete lung reexpansion and apposition of the lung to the surrounding tissues. Further studies are required in a setting that is more similar to that observed

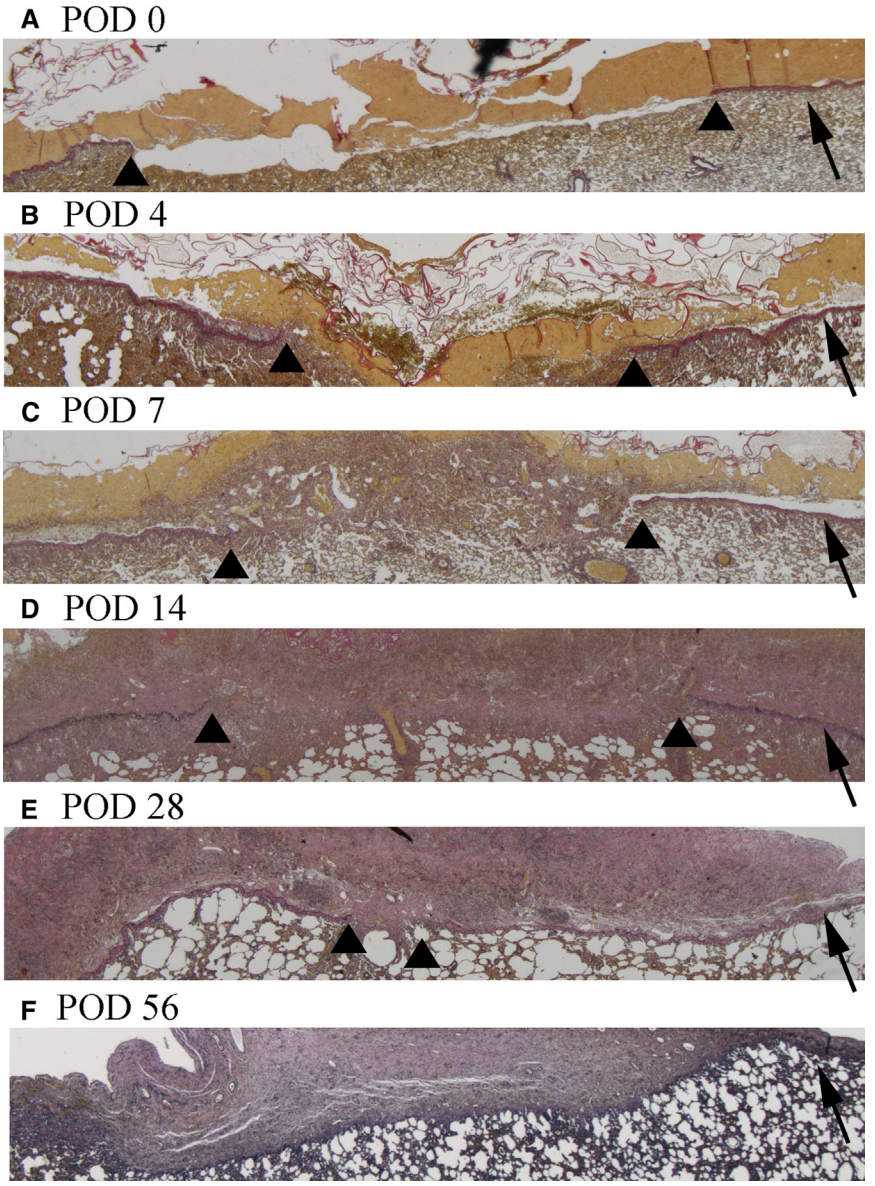

FIGURE 3. Findings of Elastica van Gieson staining. Sections stained with Elastica van Gieson visualized using a loupe (A-F). *Arrow* and *arrowheads* show elastic layer and both edges of it, respectively. Elastic layer started to regenerate from both edges on POD 28 (E) and exhibited repair by POD 56 (F). *POD*, Postoperative day.

clinically. Second, this study did not include a control group, and we assessed only a single animal at each time point. However, although the results in this study were not adequate for performing a quantitative analysis, similar results were obtained in 3 to 4 tissues per time point. Given this finding, we presumed that including additional animals would not greatly change the overall results, so we minimized the number of dogs used from the standpoint of animal welfare. These limitations should be considered when interpreting the results and conclusions of this study.

CONCLUSIONS

Our study histologically demonstrated the long-term healing processes associated with TachoSil, which is used to prevent air leakage. TachoSil safely repaired pleural defects without negatively affecting the lung parenchyma and without leaving remaining foreign material.

Conflict of Interest Statement

Authors have nothing to disclose with regard to commercial support.

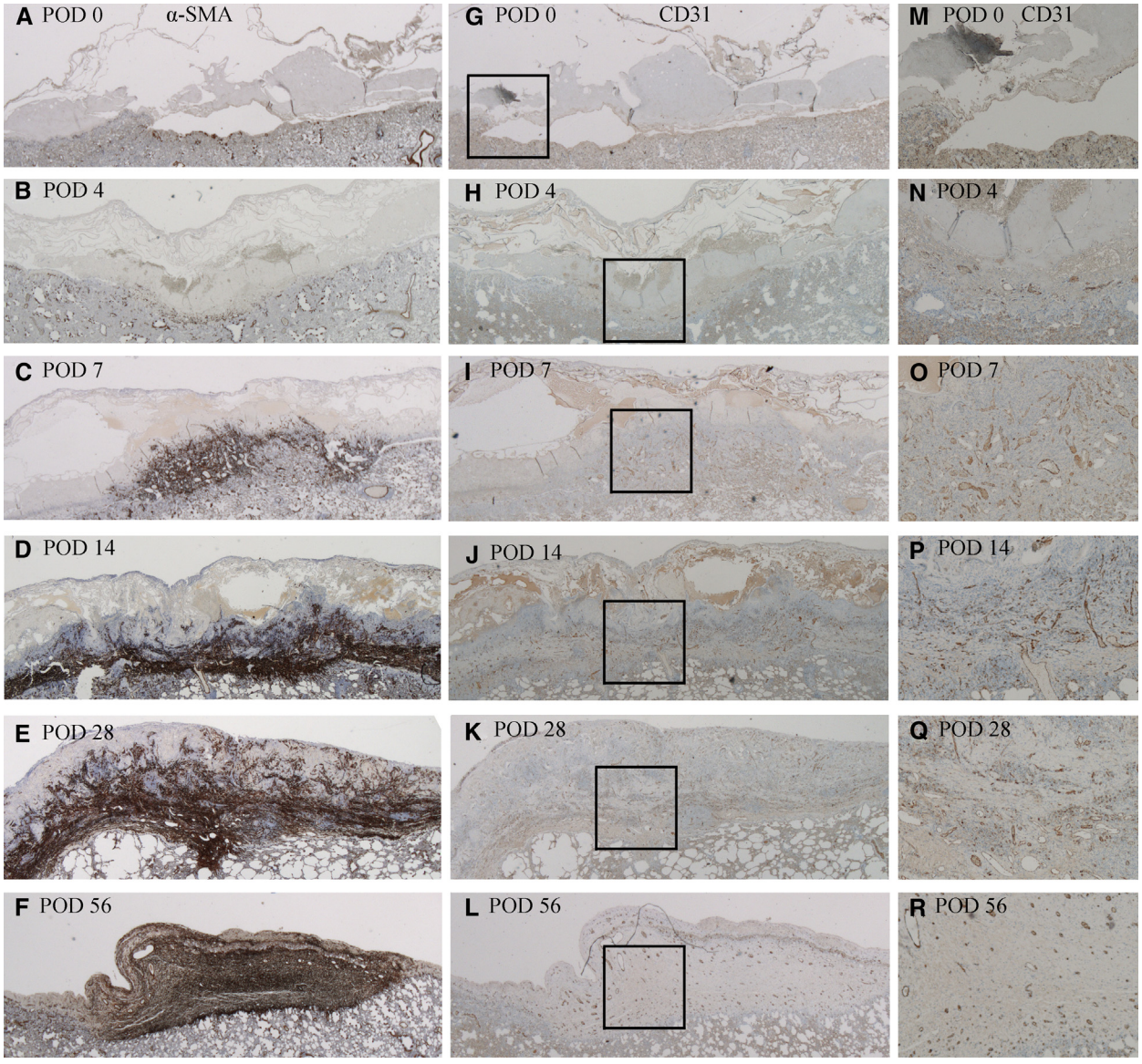

FIGURE 4. Staining of myofibroblasts and neovascular vessels. Sections stained with α-SMA (A-F) and CD31 visualized using a loupe (G-L) and CD31 stain at ×100 magnification (M-R). Myofibroblasts and neovascular vessels have formed and widely spread throughout TachoSil from POD 7 to 28, occupying the entire TachoSil on POD 56. TachoSil collagen patch coated with human fibrinogen and thrombin. *α-SMA*, α-Smooth muscle actin; *POD*, postoperative day.

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