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Original Article

Experimental Reconstruction of the Trachea with Urinary Bladder Wall

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Purpose: To investigate tracheal reconstruction with autologous bladder wall using modern refined surgical procedures.
Methods: Experiments were performed on 16 female beagle dogs. Six tracheal cartilages were resected to create a tracheal deficit, then tracheal replacement with autologous bladder wall was performed. In the first 10 dogs (first series), the transplant site was covered with pedicled omental flap. In the next six dogs (second series), we performed tracheal reconstruction without omental covering, and secured tracheal cartilages above and below the graft with sutures to prevent excessive graft stretching.
Results: No surgical mortality or lethal infection of the transplant site was encountered in either series. Complications in the first series comprised tracheal stenosis in four dogs. One dog died suddenly at 4 months postoperatively due to stent migration, so cartilage sutures were adopted in the second series. The lumen surface of the grafts was covered with squamous metaplastic epithelium. Osseous tissue was present in the submucosa of grafts, particularly prominently in areas lacking omental covering.
Conclusions: Tracheal reconstruction using bladder wall may become clinically useful. A pedicled omental covering does not appear always necessary to prevent graft necrosis and infection. Ischemic stimulation may be involved with bone formation in grafts.

Keywords: tracheal reconstruction, tracheal replacement, urinary bladder, bone formation (ossification)

Introduction

Tracheal reconstruction is an important issue in respiratory tract surgery, and is often necessary in patients with primary tracheal tumor, tracheal invasion by thyroid cancer, or tracheal stenosis as a complication after tracheostomy. Currently, the safest and most reliable method of tracheal reconstruction is end-to-end anastomosis, but the extent of resection is limited to about 6 cm.[1)] When more extensive trachea resection is necessary, permanent tracheal fistula is required.

Various biomaterials[2,3)] and artificial materials[4–6)] have been experimentally applied as tracheal replacements for tracheal reconstruction after extensive resection, but are not widely used in clinical practice. Among these materials for tracheal replacement, we have focused our attention on use of the bladder wall, as reported in 1956 and 1973.[7–9)] This tracheal replacement, as a cylinder of autologous bladder wall, is transplanted to areas of tracheal deficit. The surgical procedure is advantageous in terms of the convenience of reconstruction using autologous

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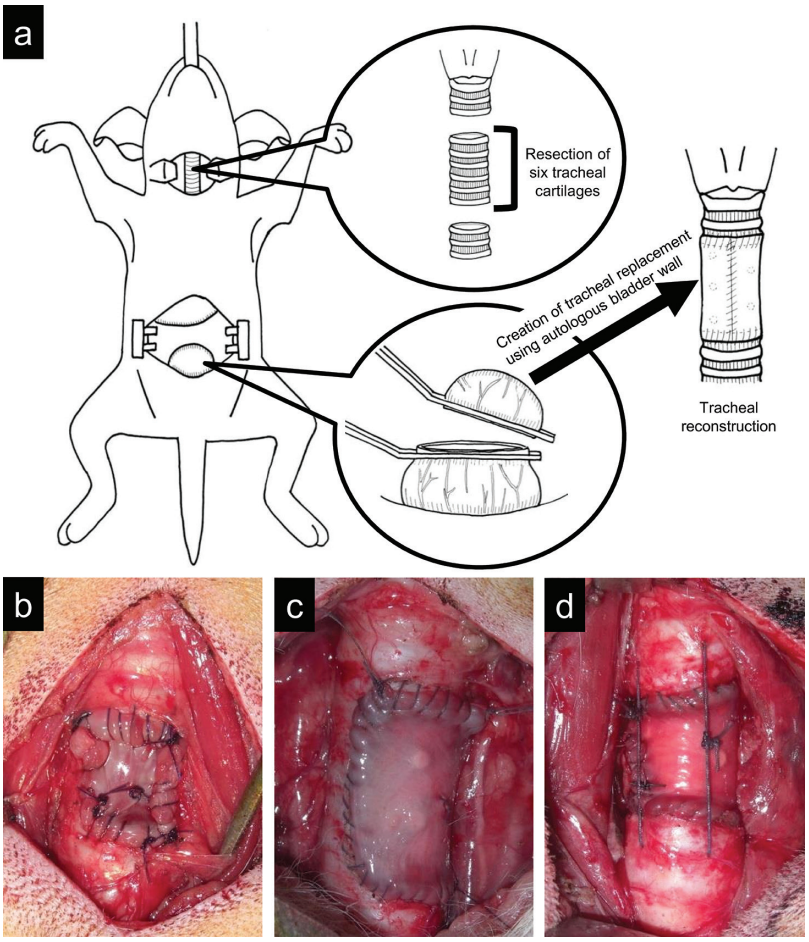

Fig. 1 Surgical procedure and operative findings. (**a**) Tracheal reconstruction was performed by transplantation of a tracheal replacement made with the bladder wall in the areas of tracheal deficit (six resected tracheal cartilages). (**b**) Patch closure of small deficit. (**c**) Semicircular tracheal replacement. (**d**) Circumferential tracheal replacement.

tissue, and moreover, ossification of the transplanted bladder wall allows the tracheal lumen to be maintained after stent removal.

Deaths due to premature loss of stents and infections were unfortunately encountered in the original studies, obviously limiting the clinical use. However, the surgical instruments and drugs used in animal experiments and the animal management environment have improved markedly over the last 40 years. We now believe that with more elaborate surgical procedures, the surgical outcomes can be greatly improved. We have therefore refined the previous surgical procedures and reinvestigated the safety and effectiveness of tracheal reconstruction using the bladder wall and its possible clinical application.

Materials and Methods

Animals

Animal care and all procedures were performed in accordance with the guidelines of the Science Council of Japan and the Guide for the Care and Use of Laboratory Animals. This study was approved by the Research Committee for Laboratory Animal Science at University of Ryukyus, Japan.

Experiments were performed on 16 female beagle dogs weighing 8.0–18.0 kg.

Anesthesia

Each dog was sedated by intramuscular injection of ketamine hydrochloride at 10 mg/kg body weight.

Table 1 First series: tracheal reconstruction using bladder wall with a pedicled omental covering (all reconstruction procedures were circumferential tracheal replacements)

Dog	Observation period (months)	Cause of death	Tracheal stenosis	Calcification on X-ray
1	54	Unknown	Severe	Indistinct
2	6	Sacrifice	Mild	Distinct
3	12	Sacrifice	None	Indistinct
4	4	Stent migration	None	*1
5	4	Sacrifice	None	Distinct
6	3	Sacrifice	Severe	Distinct
7	13	Sacrifice	Mild	Indistinct
8	12	Sacrifice	None	Indistinct
9	2	Sacrifice	None	Indistinct
10	4	Unknown	None	Distinct

*1: X-rays not performed

Endotracheal intubation and mechanical ventilation were then applied. One intravenous line was inserted into an anterior limb vein. The dog was placed in the supine position on an operating table under intravenous anesthesia with propofol (0.06–0.17 mg/kg/min).

Surgical procedure

A tracheal replacement made with autologous bladder wall was transplanted in the area of tracheal deficit in each dog (**Fig. 1a**). In the first 10 dogs (first series), the transplant site was covered with a pedicled omental flap. In the next six dogs (second series), no omental covering was used. The surgical procedure in each series is described below.

(A) First series (tracheal reconstruction with pedicled omental covering)

A midline incision was made in the abdomen, the greater omentum was resected from the greater curvature of the stomach, and a pedicled omental flap that could reach the neck, as the transplant site, was created. Next, part of the body of the bladder was resected as material to create the tracheal replacement. A longitudinal incision was then made in the anterior neck to reach the trachea, and tubular resection of six tracheal cartilages was performed to create a tracheal deficit. Artificial ventilation was changed from oral intubation to through the operative field.

A cylinder made with the obtained bladder wall was used as the tracheal replacement and placed in the tracheal deficit. This was anastomosed to the upper and lower tracheal stumps using 3-0 absorbable sutures. The bladder mucosa faced outward at this time. A silicone tracheal stent (outer diameter 18 mm, length 70 mm, Dumon tube; Novatech, La Ciotat, France) was placed as an internal stent to maintain the tracheal lumen. The pedicled omental flap that was initially created was guided retrosternally to the neck to cover the transplant site and ensure blood flow.

(B) Second series (tracheal reconstruction without pedicled omental covering)

After postoperative follow-up in the first series for 54 months, a second series was performed in six dogs without use of an omental covering. To confirm whether tracheal reconstruction could be safely performed without an omental covering, we gradually increased the transplant area, starting with patch closure of a small 1 cm × 1 cm deficit, followed by semicircular and then circumferential tracheal replacement (**Figs. 1b–1d**).

To prevent excessive stretching of the graft, the tracheal cartilages immediately above and below the graft were secured with thick absorbable suture.

A cephem antibiotic (cefpiramide sodium, 0.5 g) was administered by intravenous injection just before surgery in both series. If the surgery lasted more than 3 h, 0.5 g of cefpiramide sodium was again intravenously administered 3 h postoperatively.

After surgical wound closure and sufficient arousal of the experimental animal, the tracheal tube was removed, and the animal was returned to its cages when respiration was stable.

Endoscopy was periodically performed after surgery to examine for possible tracheal stenosis. Planned sacrifice and necropsy of the dogs were performed and radiography and histologic examination were performed when indicated.

Results

First series

Table 1 summarizes the postoperative outcomes in the first series of tracheal reconstruction. No surgical mortality, postoperative anastomotic insufficiency, or lethal infection of the transplant site was encountered. Postoperative

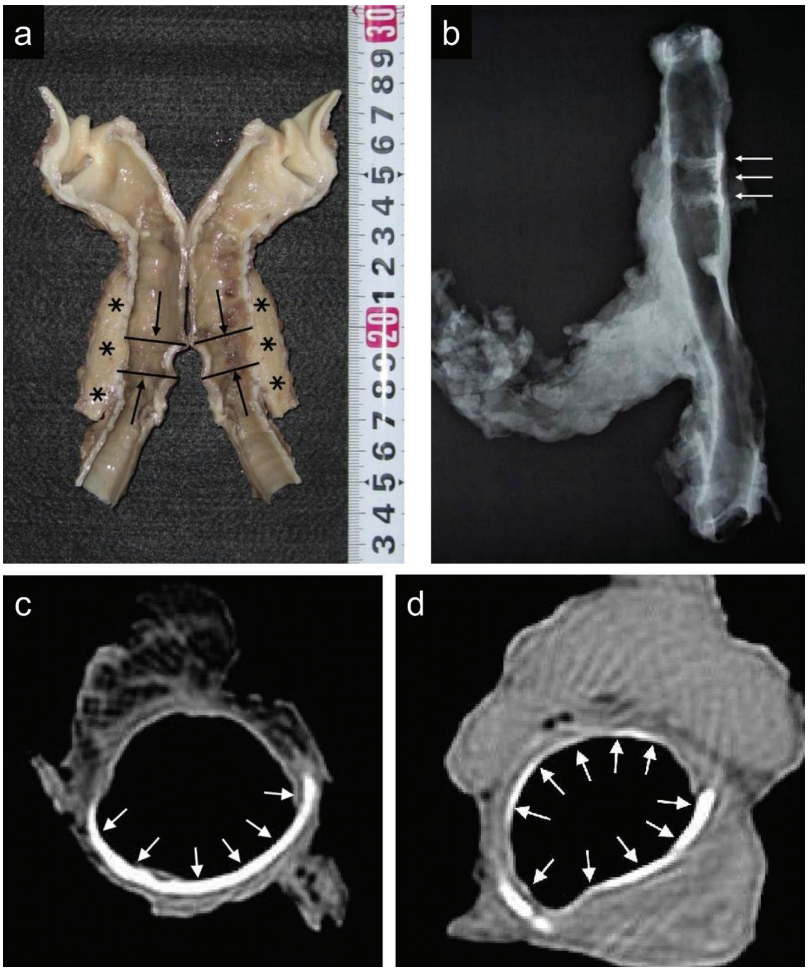

Fig. 2 (**a**) Resected specimen from the first series. Integration of the tracheal replacement (arrows) is apparent. The omental flap (asterisk) shows no necrosis and is integrated with the graft. (**b**) Radiography of the first series shows calcification (arrows) of the graft. (**c**) CT of the first series. Marked calcification (arrows) of the posterior surface is seen where the greater omentum is not in direct contact. (**d**) CT of the second series. An even distribution of calcification (arrows) is seen in the graft. CT: computed tomography

complications occurred in 4 of the 10 dogs, in the form of tracheal stenosis on postoperative endoscopy. The cause of stenosis was granulation of the tracheal lumen surface in contact with the lower end of the internal stent. Two of the 4 dogs showing tracheal stenosis were asymptomatic, but the other two dogs displayed wheezing and dyspnea. However, none of the dogs experienced respiratory failure leading to death.

One dog suddenly died during follow-up at 4 months postoperatively, and necropsy revealed suffocation due to stent migration as the cause of death.

In all cases, the bladder wall transplant graft for reconstruction of the tracheal deficit was integrated. Moreover, no necrosis was seen in the pedicled omental flap obtained from the abdomen, which integrated with the graft and surrounding tissue. The graft was not entirely covered by the greater omentum; instead, part of the posterior surface was not in contact with the greater omentum. Nevertheless, the graft was integrated without anastomotic insufficiency or infection (**Fig. 2a**).

Radiographs of resected specimens at 3, 4, and 6 months postoperatively showed calcification in the grafts (**Fig. 2b**). Computed tomography of the graft specimen showed marked calcification of the posterior surface not in direct contact with the greater omentum (**Fig. 2c**). On the other hand, radiographs of grafts at 2 months and ≥12 months postoperatively did not show calcification.

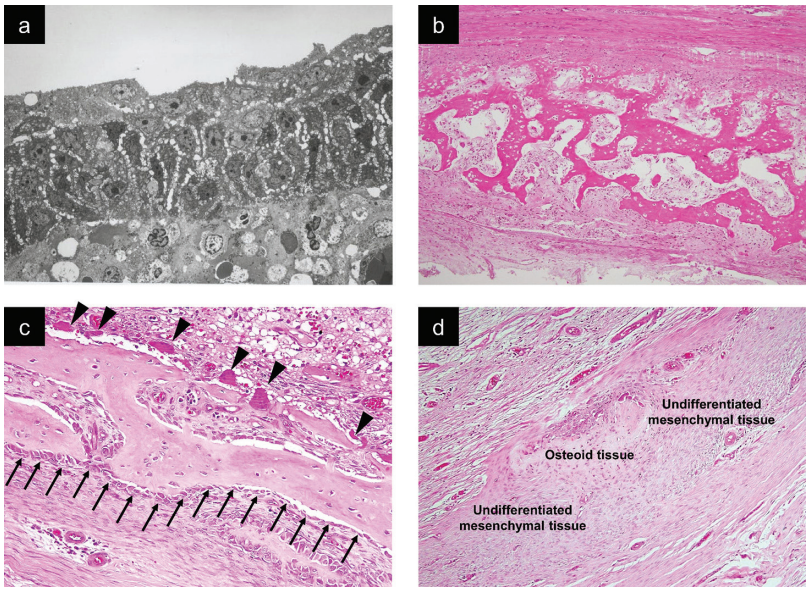

Fig. 3 Histological examination. (**a**) Electron microscopy shows neoplastic epithelium comprising stratified squamous or cuboidal epithelium and intercellular bridging. Basal cells have a continuous basement membrane. (**b**) Osseous tissue in the submucosa of the graft (hematoxylin and eosin (HE) stain, ×100). (**c**) Osteoclasts (arrowheads) on the tracheal lumen side of the osseous tissue layer, and osteoblasts (arrows) aligned outside this layer (HE stain, ×200). (**d**) Osteoid tissue is surrounded by undifferentiated mesenchymal cells. Outside of this area, well-differentiated connective tissue is apparent (HE stain, ×100).

Histological examination showed stratified metaplastic epithelium on the lumen side of the graft. Electron microscopy showed neoplastic epithelium comprising stratified squamous or cuboidal epithelium and intercellular bridging. The basal cells showed a continuous basement membrane (**Fig. 3a**). Examination of specimens at 4 and 6 months postoperatively showed a calcareous layer in the submucosa of the graft. Osteocytes were present in this calcareous layer, so this represented osseous tissue rather than simple calcification (**Fig. 3b**).

Second series

Table 2 summarizes the postoperative outcomes in the second series. All surgical procedures were successfully performed without complications such as stent migration or tracheal stenosis. Necropsy in dogs that underwent semicircular and circumferential tracheal replacement showed complete integration of the transplanted grafts.

Two dogs with semicircular replacement and one with circumferential replacement were sacrificed at 4–6 months postoperatively. Radiographs of the transplant sites showed calcification. Unlike in the first series, the calcification was evenly distributed (**Fig. 2d**). Histological examination showed findings similar to the first series, with metaplastic epithelium on the graft lumen surface and an osseous tissue layer within the connective tissue. Osteoclasts were seen on the tracheal lumen side of the osseous tissue layer, and osteoblasts were aligned outside this layer (**Fig. 3c**). Some osteoid tissue surrounded by undifferentiated mesenchymal cells was present, and well-differentiated connective tissue was seen outside this area. Continuous transitions without clear boundaries were evident between osteoid tissue, undifferentiated mesenchymal tissue, and well-differentiated connective tissue (**Fig. 3d**).

On the other hand, in one dog at 12 months after circumferential tracheal replacement, no ossification was observed.

Discussion

Tracheal reconstruction using bladder wall

Tracheal reconstruction using bladder wall was originally reported in 1956[7] and again in two papers in 1973.[8,9] These tracheal reconstructions were performed by making a cylinder in which the bladder mucosa faced outward,

Table 2 Second series: tracheal reconstruction using bladder wall, but without a pedicled omental covering

Dog	Observation period (months)	Surgical procedure	Cause of death	Tracheal stenosis	Calcification on X-ray
11	22	Patch closure	*2	None	*3
12	21	Patch closure	*2	None	*3
13	5	Semicircular	Sacrifice	None	Distinct
14	6	Semicircular	Sacrifice	None	Distinct
15	4	Circumferential	Sacrifice	None	Distinct
16	12	Circumferential	Sacrifice	None	Indistinct

*2: Living; *3: X-rays not performed; Patch closure: patch closure of deficit; Semicircular: semicircular tracheal replacement; Circumferential: circumferential tracheal replacement

then inserting a silicone stent for fixation. An advantage of this construction was ossification of the bladder wall, but deaths due to premature stent loss and infections were often reported, markedly limiting the attractiveness for clinical application. To prevent such serious complications, we altered the operative procedure from previous reports.

First, to prevent premature stent loss, we used a Dumon tube as a specialized tracheal stent, rather than the silicone elastomer (Silastic) stent applied in previous reports. The Dumon tube has studs that prevent stent migration after placement. In our first series, however, one dog still experienced stent migration and died. This stent migration was attributed to excessive stretching of the graft, so the tracheal cartilages above and below the graft was secured together with suture as an additional step in the second series. This treatment was not performed in previous reports.

As another difference from previous reports, the transplant site was covered with a pedicled omental flap to ensure blood flow around the transplant site and prevent graft necrosis and infection in the first series. However, marked ossification arose where the omental flap was not in direct contact with the graft, suggesting that contact with the pedicled omental flap might inhibit graft ossification. Therefore, to examine the possibility of ossification of the entire transplant if no omental covering were used, tracheal reconstruction was again planned, but without an omental covering. However, in tracheal reconstruction without an omental covering, we were concerned about possible graft necrosis and infection due to insufficient blood flow. We therefore started with patch closure of a small tracheal deficit, gradually increasing the transplant area in subsequent animals.

The results showed that even without omental covering, neither graft necrosis nor infection occurred, and ossification of the entire transplant was seen as expected in the second series. Our experience suggested that a pedicled omental covering was not always necessary to prevent graft necrosis and infection. Given that large numbers of infections of the transplant site have been reported in previous studies, the reason for the complete absence of infections from our study is not entirely clear. However, those previous studies were conducted more than 40 years ago, so differences in the types of antibiotics available, methods of administration, and intra- and postoperative environmental hygiene may have contributed to the absence of infections in our series.

Tracheal reconstruction without an omental covering was performed for the purpose of structural reinforcement of the graft due to widespread ossification, and the surgical procedure was simplified as a by-product.

The lumen surface of the bladder wall transplant graft was covered with metaplastic epithelium. Based on the findings of electron microscopy, the neoplastic epithelium was judged to represent squamous metaplasia. Because the bladder wall had been anastomosed with the mucosal surface facing outward (inside-out), we considered that bladder serosa had developed into squamous metaplastic epithelium.

Osseous tissue was present in the submucosa of the grafts from 4–6 months postoperatively. On the other hand, no ossification was seen at 2 months or ≥12 months postoperatively. Our findings suggest that in tracheal replacements made with bladder wall, ossification occurs at 4–6 months postoperatively, but that the resulting osseous tissue may then disappear by 12 months postoperatively.

If the circumferential osseous tissue formed in the graft remained present for a long period of time, we would remove the stent when graft ossification occurs, and stent removal contributes to the prevention of complications associated with long-term stent placement, such as granulation and infection. However, in this study, the osseous tissue

that formed had already disappeared by 12 months postoperatively, thus possibly reducing structural strength of the graft. Whether stent removal is beneficial, and the most appropriate timing for such removal, thus remain issues for further investigation. Nevertheless, tracheal reconstruction using bladder wall may be a useful provisional surgical procedure until the future clinical availability of tracheal prostheses, or in clinical settings for emergency treatment, such as when a wider tracheal resection than expected must be performed. In particular, for patch closure of a small tracheal deficit where no stent placement is required, this procedure may be clinically useful even at this stage.

Mechanism of ossification

As described in three previous reports,[7–9] ossification of the tracheal replacements made with bladder wall also occurred in our animal study. Huggins[10] investigated the mechanism of heterotopic bone formation in urothelium by transplantation of urothelium into connective tissue in adult dogs. In connective tissue just below the urothelium, ossification with bone marrow was seen, revealing a relationship between the urothelium and heterotopic bone formation. Kobayashi[11] explained the mechanism of heterotopic bone formation in transplanted urothelium as a tissue induction concept. In other words, during proliferation of the transplanted urothelium, activation and proliferation of surrounding connective tissue cells occur. During this time, conversion to osteogenic cells occurs in some fibroblast populations, intercellular hyaline material develops, bone matrix is formed, and ossification occurs.

In our study, histological examination of the grafts showed undifferentiated mesenchymal tissue between osteoid tissue in the submucosa and the surrounding connective tissue, and a continuous transition between these tissues. These findings suggest a process of dedifferentiation (blastogenesis) of fibroblasts in the graft to mesenchymal stem cells and, moreover, conversion to osteoblasts.

Our study also demonstrated definite directionality of the graft ossification. Osteoclasts were seen on the tracheal lumen side of the osseous tissue layer in the graft and a row of osteoblasts were seen outside this layer. This suggests that ossification occurs from the tracheal lumen toward the outer surface.

Etiology of ossification

Nishimura[12] ligated the renal arteries and veins in rabbits and reported a mesenchymal reaction beneath the renal pelvic epithelium and muscularis. Miyake[13] reported that ischemia of connective tissue under the urothelium and the deposition of calcium salts may be reasons for heterotopic bone formation. However, a contrary report also described ossification in areas of rich blood flow.[14] The mechanisms underlying ossification thus remain poorly understood. In our study, marked ossification of the graft was seen in areas lacking omental covering, or when no omental covering at all was used in the surgical procedure. Moreover, histological examination suggested that ossification started on the tracheal lumen side, which has a poor blood supply. We therefore believe that ischemic stimulation may be involved in this ossification.

We hypothesize that ischemic stimulation may be the reason for the ossification seen at 4–6 months postoperatively in our study, but that had disappeared by 12 months. Osseous tissue that forms in an environment of some post-transplant ischemia may move gradually from the tracheal lumen side in an outward direction during the process of remodeling. The osseous tissue may then have also disappeared as the ischemia resolved over time. However, our hypothesis requires further investigation and confirmation.

If the etiology of ossification of the grafts can be further elucidated, and a way can be found to maintain the osseous tissue for a long period of time, stent removal after circumferential tracheal replacement will be feasible. This would make tracheal reconstruction using bladder wall a more clinically useful procedure.

Conclusion

In our experimental tracheal reconstruction using autologous bladder wall in beagle dogs, graft integration was achieved without necrosis or infection. This procedure may offer a useful provisional surgical procedure until future clinical availability of a tracheal prosthesis.

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Disclosure Statement

The authors have declared that no conflicts of interest exist.

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