Effects of repeated gas sterilization on closure rates of ameroid ring constrictors in vitro

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OBJECTIVE
To determine the effect of repeated gas sterilization on rate of closure of ameroid ring constrictors in vitro.

SAMPLE
Twenty-four 3.5-mm ameroid ring constrictors.

PROCEDURES
Ameroid ring constrictors were allocated to 1 of 4 treatment groups (6/group) to undergo gas sterilization 0, 1, 5, or 10 times. After sterilization, constrictors were incubated in canine plasma at a protein concentration of 3 g/dL for 27 days. A digital camera was used to obtain images of the constrictors prior to and at various points during incubation, and lumen diameter was measured.

RESULTS
Mean ± SD percentage of lumen closure for all groups of ameroid ring constrictors combined was 85.2 ± 1.6% at day 0 (prior to plasma incubation) and 95.4 ± 0.8% at day 27. Mean lumen area was 3.64 ± 0.43 mm^2 (95% confidence interval, 2.67 to 4.77 mm^2) at day 0 and 1.32 ± 0.25 mm^2 (95% confidence interval, 0.76 to 2.04 mm^2) at day 27. None of the ameroid ring constrictors had closed completely by day 27.

CONCLUSIONS AND CLINICAL RELEVANCE
Overall closure rates for ameroid ring constrictors appeared to be unaffected by repeated gas sterilization up to 10 times. Findings suggested that veterinary surgeons can resterilize ameroid ring constrictors up to 10 times with confidence that ring properties would remain suitable for clinical use. (*Am J Vet Res* 2016;77:84–87)

Surgical management of congenital extrahepatic portosystemic anomalies is usually achieved by complete or partial occlusion of the shunting vessel.[1–3] The goal of surgical treatment is to redirect portal blood through the liver to palliate the clinical signs of hepatic encephalopathy.[4] Complete occlusion of the shunt has been associated with a better long-term prognosis than partial ligation or medical management, and various techniques have been used to gradually and completely occlude the shunt without increasing the risk of acute portal hypertension.[5–9] Although multiple surgical techniques exist to partially occlude the shunting vessel, the 2 most common approaches are partial ligation with a cellophane band and use of an ameroid ring constrictor, both of which provide gradual shunt occlusion.[1,9,10]

Ameroid ring constrictors consist of a slotted stainless steel ring surrounding an inner compressed casein band with a key mechanism, permitting the device to be placed around a vessel. Ameroid is a hygroscopic casein derivative and undergoes rapid volumetric expansion that plateaus in 60 days.[11,12] As the casein gradually absorbs fluids and expands, the inner diameter (lumen) closes and constricts the shunting vessel.[8] The ameroid also triggers an inflammatory reaction around the shunt that contributes to closure[13] and possibly thrombosis[14] of the shunt. The device is available in multiple sizes (diameters of 3.5, 5, 6.5, 7, 8, and 9 mm). The 3.5- and 5-mm-diameter rings are used most commonly in the management of single extrahepatic portosystemic shunts in dogs.[4]

One consideration regarding closure of ameroid ring constrictors is the intraoperative selection of constrictor size and need to resterilize the unused constrictor. When a surgeon elects to place an ameroid ring constrictor on the shunting vessel, a decision pertaining to the size of the device may need to be made intraoperatively.[15] Because this decision must be made at the time of surgery, a surgeon must have several constrictor ring sizes available. Thus, unused ameroid ring constrictors must be resterilized and returned to stock until the next surgery. However, it is unknown whether repeated sterilization of these restocked constrictors affects the rate of closure or the likelihood that they will close sufficiently.[a] When unused constrictors are not resterilized after 365 days, they must be discarded, which results in a monetary

ABBREVIATION
CI Confidence interval

loss. Currently, gas sterilization is the only means of sterilization for ameroid ring constrictors, and the temperature required to achieve sterilization (60°C) may denature the casein protein within the constrictor.[16] Currently, surgeons who resterilize these constrictors risk not knowing whether the casein protein within the ring will have its closure function attenuated by reprocessing. The manufacturer states that the constrictors can only be gas sterilized twice and have a shelf life of 1 year.[a]

The purpose of the study reported here was to evaluate the effects of repeated gas sterilization on the rate and degree of lumen closure of ameroid ring constrictors in vitro. We hypothesized that rate and degree of lumen closure would not be influenced by repeated gas sterilization in vitro.

Materials and Methods

Twenty-four 3.5-mm ameroid ring constrictors[b] were obtained in packaging direct from the manufacturer and allocated to 1 of 4 treatment groups (6/group) to be gas sterilized 0 (control group), 1, 5, or 10 times and subsequently incubated in canine plasma to assess rate and degree of constriction. For gas sterilization, constrictors were placed in an ethylene oxide gas sterilizer[c] for 360 minutes. A period of 24 to 96 hours separated sterilization sessions.

After treatment, ameroid ring constrictors were incubated in accordance with a previously reported in vitro culture method.[4] Briefly, plasma was aseptically obtained from packaged plasma provided by multiple dog donors for use in the plasma bank of the veterinary teaching hospital at Mississippi State University. All plasma was diluted with saline (0.9% NaCL) solution and maintained at a protein concentration of 3 g/dL, as confirmed with a refractometer.[d] One milliliter of 0.1% streptomycin solution and 1 mL of 0.05% amphotericin B solution were added to the plasma. Each treatment group of constrictors was placed in a standard 10 X 100-mm sterile polystyrene Petri dish containing treated plasma, and Petri dishes were incubated in a standard cell culture incubator for 27 days at 37°C and 5% CO_2. Plasma was changed every 48 hours.

A digital camera[e] was used to photograph the ameroid ring constrictors when received from shipping and during incubation at 0 (prior to incubation), 1, 6, 11, 16, 21, and 27 days. A standard photography ruler was used to ensure accurate, equidistant measurements. The camera was held perpendicular to each Petri dish at a consistent height of 30 cm. The same zoom lens (35 mm) was used for each photograph. The same image quality settings were used for each photograph (International Standards Organization 220; focal length, 2.8; shutter speed, 1/30). Total (outer) area and lumen (inner) area of each constrictor were measured in triplicate at each assessment point with imaging software.[f] Each set of 3 measurements was then averaged and used for analysis. Differences in areas were calculated, averaged, and recorded as percentage closure (total area – lumen area)/total area X 100).

Statistical analysis

Data were summarized as mean ± SD. Two-factor ANOVA with 1 between-subjects factor treatment (number of sterilization sessions) and 1 within-subjects factor (time or day of measurement) was used to compare treatment groups of ameroid ring constrictors.[g] The outcome variable was the difference between total and lumen areas of the constrictor, divided by the total area. Because repeated measurements of each constrictor were made over time, the constrictor was included in the model as a random effect. A treatment by time interaction term was also included. Because the interaction term was significant, differences between treatment means on each day were estimated by calculating least square mean value. The 95% CIs of mean differences were calculated, and the importance of significant differences was assessed on this basis.[17] End points of these CIs were used to determine whether the estimated differences were clinically important.[17] Residuals from the fitted model were examined and tested for normality by means of the Shapiro-Wilk test. Because many pairwise comparisons of means on different days were made, the false discovery rate approach was used to control for multiple testing.[18] For all tests, values of $P < 0.05$ after adjustment were considered significant.

Results

Differences between mean lumen areas measured prior to and after 27 days of plasma incubation were significant for all treatment groups of ameroid ring

Table 1—Mean ± SD initial* and final lumen areas of ameroid ring constrictors that were gas sterilized 0, 1, 5, or 10 times (n = 6/group) and incubated in canine plasma for 27 days.

No. of sterilization sessions	Initial area (mm^2)	Final area (mm^2)	Percentage closure†	Difference between initial and final area (%)	*P* value
0	3.73 ± 0.49	1.52 ± 0.26	95	59	< 0.005
1	3.28 ± 0.37	1.21 ± 0.15	96	63	< 0.005
5	3.84 ± 0.42	1.34 ± 0.21	95	65	< 0.005
10	3.26 ± 0.44	1.21 ± 0.24	95	63	< 0.005
Pooled data	3.53 ± 0.43	1.32 ± 0.22	95	63	< 0.005

*Initial area was measured prior to incubation in canine plasma. †Percentage closure was calculated as follows: (total constrictor area – lumen area)/total constrictor area X 100.

Values of $P < 0.05$ after adjustment for multiple tests were considered significant.

Table 2—Mean ± SD percentage closure of ameroid ring constrictors that were gas sterilized 0, 1, 5, or 10 times (n = 6/group) at various points of subsequent plasma incubation.

No. of sterilization sessions	Day 0*	Day 1	Day 6	Day 11	Day 16	Day 21	Day 27
0	84.5 ± 1.3[a]	87.6 ± 1.1	90.1 ± 0.6	92.4 ± 0.6[a]	92.9 ± 0.7	94.6 ± 0.9	95.2 ± 0.8
1	85.6 ± 1.1[a,b]	87.6 ± 1.2	89.8 ± 1.4	90.0 ± 0.8[b]	92.2 ± 0.7	93.9 ± 0.5	95.7 ± 0.6
5	84.6 ± 1.4[a,b]	88.4 ± 1.3	90.5 ± 0.7	91.2 ± 0.9[a,b]	92.4 ± 0.6	93.2 ± 0.8	95.0 ± 0.8
10	86.1 ± 1.8[b]	87.7 ± 1.1	89.7 ± 1.0	91.1 ± 1.1[a,b]	92.2 ± 1.3	93.3 ± 1.1	95.6 ± 1.0

*Day 0 represented prior to incubation.

[a,b]Within a column, values with different superscript letters differ significantly (adjusted $P < 0.05$). However, the magnitudes of these differences (0.016 mm^2 on day 0 and 0.024 mm^2 on day 11) were deemed clinically unimportant.

constrictors (gas sterilized 0, 1, 5, or 10 times; **Table 1**). Mean ± SD lumen area was 3.64 ± 0.43 mm^2 (95% CI, 2.67 to 4.77 mm^2) at day 0 and 1.32 ± 0.25 mm^2 (95% CI, 0.76 to 2.04 mm^2) at day 27. Mean percentage closure (based on the difference between total and lumen areas of the constrictor, divided by total area) for all treatment groups combined was 85.2 ± 1.6% at day 0 (prior to incubation) and 95.4 ± 0.8% at day 27.

On days 0, 1, 6, 11, 16, 21, and 27 of plasma incubation, mean lumen area for all treatment groups combined was 3.64 ± 0.43 mm^2 (95% CI, 2.67 to 4.77 mm^2), 2.93 ± 0.29 mm^2 (95% CI, 2.41 to 3.68 mm^2), 2.32 ± 0.23 mm^2 (95% CI, 1.87 to 3.27 mm^2), 2.36 ± 0.30 mm^2 (95% CI, 1.82 to 3.23 mm^2), 1.89 ± 0.23 mm^2 (95% CI, 1.45 to 2.60 mm^2), 1.64 ± 0.25 mm^2 (95% CI, 1.04 to 2.39 mm^2), and 1.32 ± 0.25 mm^2 (95% CI, 0.76 to 2.04 mm^2), respectively. Significant (adjusted $P < 0.05$) differences in mean percentage closure were identified between some treatment groups on days 0, 11, and 21 of plasma incubation **(Table 2)**. However, given that the largest of these differences was only 0.024 mm^2 (95% CI, 0.015 to 0.034 mm^2), the magnitudes of the differences were deemed not clinically important. No significant ($P > 0.869$) difference was identified among treatment groups in percentage closure over time.

Discussion

In the study reported here, none of the ameroid ring constrictors completely closed by 27 days after incubation in canine plasma; mean percentage closure was 95% for all treatment groups, regardless of the number of times constrictors were gas sterilized. Additionally, mean lumen area was similar for all groups at the beginning (day 0) and again at the end of the 27-day period. These findings supported those of a previous study[4] in which percentage closure of ameroid ring constrictors increased with increasing duration of plasma incubation. In that study,[4] high plasma protein concentrations resulted in rapid closure of the lumens of ameroid ring constrictors, and constrictors incubated in canine plasma at a concentration of 1.5 g/dL closed to a larger overall lumen diameter (ie, smaller percentage closure) than did constrictors incubated in canine plasma at a concentration of 3 and 6 g/dL. Investigators therefore concluded that the final diameter of the ameroid lumen was a function of time and protein concentration. We obtained similar results in that a plasma protein concentration of 3 g/dL resulted in large percentage closure. The final lumen size of 1.32 mm^2 achieved after 27 days of plasma incubation in the present study would be reasonable for in vivo application, given the supposition that shunts would likely achieve functional closure because of inflammatory thrombosis.[19]

An unknown variable in the present study was the period the ameroid ring constrictor would be stored on the shelf, as this was beyond the scope of this study. We were unable to identify any reports that indicate the casein component of ameroid ring constrictors becomes denatured when constrictors are stored on the shelf at room temperature (approx 20° to 25°C). To the authors' knowledge, the shelf life of the casein after gas resterilization has also not been reported. Because the ameroid ring constrictors in the present study were purchased directly from the manufacturer and then used, the devices spent little time on the shelf. Additional studies may be warranted to ascertain the effects on percentage closure of extended shelf storage at room temperature alone or in combination with repeated sterilization.

For the plasma incubation stage of the present study, canine plasma samples from multiple donors were pooled and diluted to a protein concentration of 3 g/dL, whereas in the previous study,[4] plasma used for incubation originated from 1 donor dog. It is unclear whether use of pooled plasma would have a different effect on the ameroid ring constrictors than would use of plasma from 1 dog, but this was considered unlikely given that our results were similar to those of the previous study[4] with regard to closure.

Even with the modest sample size of 6 constrictors/treatment group, some significant differences were found among the groups. However, the largest of these differences was only 0.024 mm^2 (95% CI, 0.015 to 0.034 mm^2). Even at the upper limit of the estimate (0.034 mm^2), this magnitude of difference would be unlikely to be of clinical importance.

The lack of significant differences among the 4 treatment groups after 27 days of plasma incubation suggested that repeated gas sterilization up to 10 times had no effect on percentage closure or overall closure rate and that such constrictors could be resterilized repeatedly in clinical practice with confidence that their closure properties would not change. A larger sample size might have resulted in more differences attaining significance. However, the numeric values would generally be even smaller

than the differences observed here and thus would not be clinically important. The finding that the constrictors did not close completely after 27 days of treatment in vitro has been substantiated in other studies[4,10,13] and illustrated the need for proper size matching of constrictors to the shunting vessel to minimize potential incomplete occlusion once the lumen has closed to its fullest extent.

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Footnotes

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