In vitro expansion patterns of ameroid ring constrictors

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Objective—To evaluate and quantify in vitro dimensional changes of ameroid ring constrictors (ARCs) with and without the outer stainless steel ring in place over time.

Sample Population—12 ARCs (5.0-mm diameter).

Procedures—6 ARCs were immersed in canine plasma baths for 34 days without the stainless steel outer ring in place (group N), and 6 ARCs were immersed in canine plasma baths with the stainless steel outer ring in place (group R). The ARCs were digitally imaged at day 0, daily for the first 10 days, then on days 14, 20, 27, and 34. Inner diameter, outer diameter, luminal area, and height were measured for each time point. Volume and weight of rings were obtained at the start and completion of the study.

Results—The inner diameter, outer diameter, and luminal area were significantly different between the 2 groups over the course of 34 days. The inner diameter and luminal area of the R-group constructs did not change significantly, while the inner diameter, outer diameter, luminal area, and height of N-group constructs all significantly increased over the course of the study.

Conclusions and Clinical Relevance—R-group constructs had insignificant centripetal swelling without ring closure, whereas N-group constructs had significant generalized centrifugal expansion. Results of this study indicated that the outer stainless steel ring of an ARC may not be necessary for attenuation and closure of some single extrahepatic portosystemic shunts. (*Am J Vet Res* 2008;69:1520–1524)

Portosystemic shunts are vascular anomalies that divert portal blood away from the liver and into the systemic circulation.[1,2] They may be congenital or acquired.[1,2] Congenital, single, extrahepatic portosystemic shunts are the most common category in dogs and cats with a high incidence in certain breeds of dogs.[1,2]

Up to 68% of dogs with congenital portosystemic shunts will develop portal hypertension when surgically treated with acute shunt ligation.[3,4] Gradual occlusion of the aberrant vessel is preferred, thereby allowing the cardiovascular and nervous systems to adapt to changes in portal blood flow. This reduces the risk of developing acute portal hypertension or perioperative seizures.[5] Various methods have been described to achieve gradual attenuation and ultimate closure of the shunt vessel.[1–7]

One of the most commonly used methods for gradual attenuation is the use of an ARC. Ameroid is a casein derivative that undergoes rapid volumetric expansion that plateaus in 60 days.[4–11] The ameroid core is surrounded by an outer stainless steel ring. Occlusion likely results from a combination of the following

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Abbreviation

ARC	Ameroid ring constrictor

3 mechanisms: reduction in external vessel diameter caused by centripetal swelling of the ameroid, fibroblastic response within the vascular wall at the site of application, and ultimate formation of intraluminal thrombi arising concentrically from the intima.[5,9] Factors including size, shape, encasement of the ameroid ring, and the type and temperature of the surrounding fluid modify the expansion pattern.[7] More recently, the rate of constriction has been shown to be influenced by the protein concentration of the surrounding fluid.[12] Total luminal obliteration of a portosystemic shunt with these devices takes 4 to 5 weeks.[5] This has been documented via measurement of shunt fraction by use of transcolonic portal scintigraphy.[5,13]

Despite the benefits associated with ARC use, compared with acute, complete ligation, the use of ARCs is not entirely benign. In a retrospective study by Mehl et al,[14] 80% of owners reported an excellent outcome despite 21% of the dogs being diagnosed with persistent shunting in patients in which an ARC was used for shunt attenuation.[14] A mortality rate of 9% was also reported.[14] It has been proposed that acute kinking of the shunt vessel after ARC placement could account for increased morbidity and mortality rates in a manner similar to acute suture ligation of the shunt vessel, especially in smaller patients.[10,15] Two main theories regarding the cause of vascular kinking are the overzealous perivascular dissection and the effect of the relative high weight of the ARC upon the small and compliant vessel.

The outer stainless steel portion of the ring accounts for a large percentage of the weight of the ARC. Because the vessels are low-velocity and low-pressure vessels, changes in the flow rate and diameter can lead to a loss of normal laminar blood flow. Poiseuille's law states that the laminar flow rate of an incompressible fluid along a cylinder is proportional to the fourth power of the radius of the cylinder. Therefore, temporary kinking or flattening of a shunt vessel can lead to a dramatic change in flow turbulence, which can in turn lead to larger and more abrupt thrombogenesis. These events would ultimately lead to a rapid or acute closure of the shunt vessel rather than the desired gradual and progressive one.

By removing the outer stainless steel ring, the weight of the ARC implant is greatly reduced. In smaller patients, some veterinary surgeons will place the ARC without the outer stainless steel portion in place to reduce the weight of the ring. Theoretically, this may minimize the potential risk of shunt vessel kinking. To our knowledge, the effects of removing the stainless steel ring on ameroid expansion pattern and the weights of the constrictor components have not been reported.

The purpose of the study reported here was to evaluate and quantify dimensional changes of ARCs over time in vitro with and without the outer stainless steel ring in place. We hypothesized that changes in measured dimensional variables would not be different between ARCs with the stainless steel outer ring in place versus those without the stainless steel outer ring.

Materials and Methods

Procedures—Plasma was harvested from a blood sample obtained from a healthy male dog that was subsequently euthanatized by lethal injection for an unrelated study approved by the Iowa State University Committee on Animal Care. The plasma was analyzed and had a protein concentration of 6.0 g/dL, a glucose concentration of 106 mg/dL, and a BUN concentration of 16 mg/dL. Electrolytes were also analyzed. Plasma sodium concentration was 145 mEq/L, and the potassium concentration was 4.1 mEq/L.

Twelve 5-mm diameter ARCs[a] were randomly assigned to 2 groups. The first group (n = 6) was aseptically placed in canine plasma with the outer stainless steel ring in place (group R). The second group (n = 6) of ARCs was placed in the same canine plasma, but the outer ring was removed (group N). All 12 rings were placed in the identical sterile plasma bath separated by polyvinylchloride chambers to facilitate constrictor identification and prevent intermingling of the constrictors. The plasma was aseptically changed twice weekly to prevent microbial colonization. Samples of plasma were not submitted for bacterial culture at the time of the exchanges. The plasma was incubated in a water bath[b] at 39°C.

Before immersion into the plasma bath, each ameroid core and corresponding outer stainless steel ring was weighed by use of a precision scale.[c] A digital camera[d] was rigidly mounted 16 cm above a platform. Each ring was placed on this platform and photographed twice at a point perpendicular to the camera at orthogonal views at day 0, daily for the first 10 days, then on days 14, 20, 27, and 34. To assure that no distortion occurred as a result of potential minute movement of the camera during the course of the experiment, a precision digital caliper[e] was included in the field of the photograph to serve as a digital measurement reference. Upon completion of the experiment, each ameroid core and corresponding outer stainless steel ring was weighed again by use of the same precision scale. A public domain computer image program[f] was used to obtain measurements of inner diameter, outer diameter, and heights of each ameroid core for each time point. Luminal area was calculated by use of the equation πr^2, where r = half of the inner diameter. Volumes of the ameroid cores were obtained at the start and upon completion of the study. The volume was calculated by use of the equation $\pi h(r_o^2 - r_i^2)$, where r_o = half of the outer diameter, r_i = half of the inner diameter, and h = height.

Statistical analysis—Data were analyzed by use of a commercially available software program.[g] Data were inspected for outliers and distributional assumptions by use of summary statistics (means, medians, SDs, and histograms). A repeated-measures ANOVA was used to assess group differences. Additionally, paired *t* tests were used to compare changes (from day 0 to day 34) within a group over time for each variable. Values of $P < 0.05$ were considered significant. Results are reported as mean ± SD.

Results

Three of the 6 ameroid cores in R-group constructs sheared off above the outer stainless steel ring between day 27 and day 34 evaluations. As such, measurements from these ARCs were not included in the final day 34 analyses. Therefore, day 34 values for R-group constructs represent 3 ARCs, compared with 6 for N-group constructs and all prior measurements. No significant differences were found in the measured dimensions between the 2 groups at the start of the study.

At day 0, the mean ± SD inner diameter was 5.10 ± 0.07 mm for N-group constructs and 5.14 ± 0.03 mm for R-group constructs. At day 34, the mean inner diameter for N-group constructs was 6.77 ± 0.04 mm and that for R-group constructs was 4.60 ± 0.48 mm. Over the course of the study, the mean inner diameter for N-group constructs was significantly ($P < 0.001$) larger than for R-group constructs. When comparing day 34 mean inner diameter with day 0 mean inner diameter for N-group constructs, values were significantly ($P < 0.001$) different. The difference between the day 34 value and the day 0 value for R-group constructs, however, was not significant ($P = 0.20$; **Figure 1**).

At day 0, the mean outer diameter was 9.82 ± 0.02 mm for N-group constructs and 9.86 ± 0.04 mm for R-group constructs. At day 34, the mean outer diameter for N-group constructs was 12.95 ± 0.07 mm and that for R-group constructs was 11.79 ± 0.52 mm. Over the course of the study, the mean outer diameter for N-group constructs was significantly ($P < 0.001$) larger than for R-group constructs. When comparing day 34 mean outer diameter with day 0 mean inner diameter for N-group constructs, the values were significantly ($P < 0.001$) different. The difference between the day 34 value and the day 0 value for R-group constructs was significant ($P = 0.02$; **Figure 2**).

At day 0, the mean luminal area was 20.16 ± 0.47 mm² for N-group constructs and 20.21 ± 0.45 mm² for R-group constructs. At day 34, the mean luminal area for N-group constructs was 35.01 ± 0.59 mm and that for R-group constructs was 17.18 ± 5.30 mm. Over the course of the study, the mean luminal area for N-group constructs was significantly ($P < 0.001$) larger than for R-group constructs. When comparing day 34 mean luminal area with day 0 mean luminal area for N-group constructs, values were significantly ($P < 0.001$) different. The difference between the day 34 value and the day 0 value for R-group constructs was not significant ($P = 0.437$; **Figure 3**).

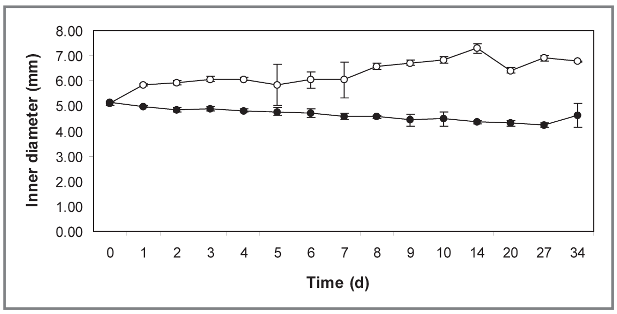

Figure 1—Mean ± SD inner diameter versus time of ARCs with (black squares; n = 6) and without (white circles; 6) the outer stainless steel ring in place.

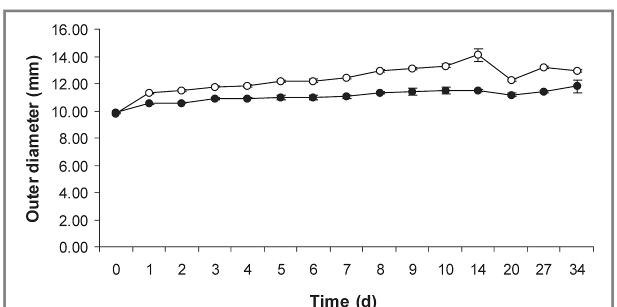

Figure 2—Mean ± SD outer diameter versus time of ARCs with (black squares; n = 6) and without (white circles; 6) the outer stainless steel ring in place.

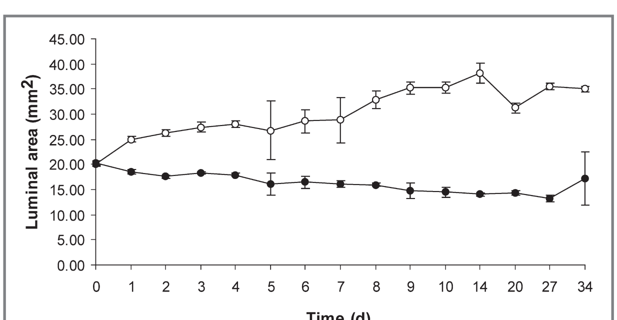

Figure 3—Mean ± SD luminal area versus time of ARCs with (black squares; n = 6) and without (white circles; 6) the outer stainless steel ring in place.

At day 0, the mean height was 5.45 ± 0.05 mm for N-group constructs and 5.52 ± 0.01 mm for R-group constructs. At day 34, the mean height for N-group constructs was 7.30 ± 0.09 mm and that for R-group constructs was 8.21 ± 0.22 mm. There was not a significant difference ($P = 0.581$) between the 2 groups over the course of the study period. When comparing day 34 mean height with day 0 mean height for N-group constructs, values were significantly ($P < 0.001$) different. The difference between the day 34 value and the day 0 value for R-group constructs was significant ($P < 0.001$; **Figure 4**).

Ameroid cores in R-group constructs had a mean volume of 307.55 mm³ on day 0 and 759.72 mm³ on day 34. Ameroid cores in N-group constructs had a mean volume of 301.76 mm³ on day 0 and 698.01 mm³ on day 34. A significant difference in mean volume was not found between the 2 groups on day 0 ($P = 0.101$) or on day 34 ($P = 0.264$). From day 0 to day 34, R-group constructs had a 247% increase in volume and N-group constructs had a 231% increase in volume, representing a 6% difference between the gain in volume of the 2 groups.

At day 0, the mean combined weight of the ameroid core and the outer stainless steel ring was 1.348 g (range, 1.338 to 1.364 g). The mean weight for N-group constructs was 1.348 g (range, 1.344 to 1.351 g) at day 0 and 1.349 g (range, 1.338 to 1.364 g) for R-group constructs. The mean weight of the ameroid cores for all 12 rings was 0.465 g (range, 0.456 to 0.470 g), accounting for a mean of 34.5% (range, 33.9% to 35.0%) of the total weight of the ARCs. For N-group constructs, the mean weight of the ameroid core at day 0 was 0.4635 g (range, 0.456 to 0.468 g), accounting

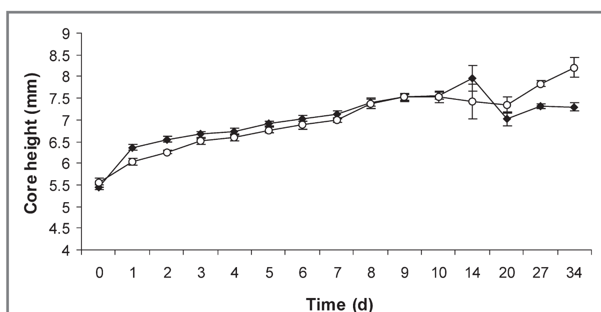

Figure 4—Mean ± SD ameroid core height versus time of ARCs with (black squares; n = 6) and without (white circles; 6) the outer stainless steel ring in place.

Table 1—Mean dimensional measurements of ARCs with (group R) and without (group N) the outer stainless steel ring in place.

Variable	N-group constructs (n = 6) Day 0	N-group constructs (n = 6) Day 34	R-group constructs (n = 6) Day 0	R-group constructs (n = 6) Day 34
Internal diameter (mm)	5.10	6.77	5.14	4.60
Outer diameter (mm)	9.82	12.95	9.86	11.79
Luminal area (mm²)	20.16	35.01	20.21	17.18
Height (mm)	5.45	7.30	5.52	8.21
Volume of casein (mm³)	301.44	698.73	306.95	758.10
Weight of casein (g)	0.46	0.99	0.47	1.01

for a mean of 34.4% (range, 33.9% to 34.7%) of the total weight of the ARCs. For R-group constructs, the mean weight of the ameroid core at day 0 was 0.467 g (range, 0.463 to 0.470 g), accounting for a mean of 34.6% (range, 34.4% to 35.0%) of the total weight of the ARCs. The mean weight of the ameroid cores for N-group constructs at day 34 was 0.986 g (range, 0.967 to 1.002 g), representing a 213% increase from day 0. The mean weight of the ameroid cores for R-group constructs at day 34 was 1.014 g (range, 0.965 to 1.098 g), representing a 217% increase from day 0. The stainless steel outer ring accounted for 65.5% of the total weight of the ARCs at day 0 and 46.9% at day 34 **(Table 1)**.

Discussion

Results of our study revealed that there was not a significant change in the luminal diameters of the rings in R-group constructs from day 0 to day 34, thus refuting our hypothesis. However, the inner ring diameter, outer ring diameter, and the area of the central lumen of the ARCs were significantly different between R-group constructs and N-group constructs over the course of the 34-day study period. These variables all increased over time in N-group constructs. These results would suggest that reduction in external vessel diameter because of centripetal swelling of the ameroid contributes little to closure of the vessel. It would be likely that perivascular inflammation and fibrosis are more important causes of vascular occlusion.[4] The low carbon version of 316-grade (ie, 316L) stainless steel of the external ring is generally considered biologically inert and nonbioreactive.[16] Its removal should therefore have no obvious impact on the reactivity of the host tissue toward the implant.

Outer diameters of rings placed without the outer stainless steel rings in place did increase. Without the outer ring, there is no obvious mechanical constrain to such expansion. It is unknown whether such outward core expansion may allow for the ultimate failure of shunt vessels closure. Alternatively, it may be advantageous in further allowing for a slow and gradual shunt closure. What is interesting, however, was that the outer diameters of the rings placed in baths with their respective outer stainless steel rings in place did undergo some minor degree of expansion. The mean outer diameters for R-group constructs significantly ($P = 0.02$) increased from 9.86 mm to 11.79 mm. It was observed that the casein portion of the ARCs did swell over the stainless steel portions; however, it cannot be fully determined as to whether there was any outward deformation of the stainless steel rings at this time. This outward expansion of the ameroid cores was observed. It is possible that this finding was subject to a type II statistical error arising from the limited number of rings placed in plasma baths in this study.

It is of particular interest to note that 50% of the ARC in R-group constructs underwent shearing of the casein. The shearing occurred between day 27 and 34 in all instances. The ARC measurements for this study were taken from digital images rather than by actual measuring of the ARC, and as such, data from these constructs could not be included in the final analyses. This phenomenon (ie, shearing of the casein) has not been reported. In a similar study[12] investigating ARC dimensional properties in vitro, measurements were only made until day 27. Perhaps shearing of the casein may have occurred in that study[12] had the study period been extended beyond day 27.

Another difference in our study design, compared with that of a previous study,[12] is that an antimicrobial agent was not added to the plasma bath. Instead, the plasma was changed twice a week to prevent contamination. This decision was made because the effect of antimicrobial agents on the rate of ARC function has not been reported. Bacterial culture of the plasma baths after each change could have been used to confirm the absence of bacterial contamination.

Limitations of our study are predominantly related to a type II statistical error associated with the limited number of ARCs tested. There are certain data points with relatively larger than expected variances. This phenomenon could have been diminished or removed by having larger numbers. This effect is even more pronounced for R-group constructs, where 50% of the constructs underwent shearing of the outer stainless steel ring. Measurement errors and lack of statistical power together may have played a role. As a result, a large number of ARCs should be tested.

The volumes in this study were obtained retroactively by use of the equation $\pi h(r_o^2 - r_i^2)$, where r_o = half of the outer diameter and r_i = half of the inner diameter. Such a calculation is a rough estimate because the rings are not true cylinders, especially after irregular casein expansion. This method of obtaining the volume of the ARCs in combination with limited numbers of tested constructs also predisposes to error. Ideally, volumes of the constructs could have been obtained prospectively by use of fluid displacement.

In addition to measuring and evaluating the linear dimensions of the rings, weights of the rings at the beginning and end of the study were measured. Results of our study revealed that 65.5% of the weight of the ARC is concentrated in the stainless steel outer ring. As suggested previously, having a more concentrated weight at the periphery of a ring may further predispose to rotation of the ring and sagging of the shunt vessel with subsequent vessel occlusion. If, in fact, the stainless steel outer ring is less integral to the whole process of the intended gradual shunt occlusion, then its removal should be benign. Its removal would furthermore be of potential benefit in small patients. Although not yet incorporated into a clinical prospective trial, it is our impression that patients that have had ARCs placed without the stainless steel outer portion do seem to improve clinically and have resolution of serum chemistry abnormalities associated with portosystemic shunting.

In conclusion, ARCs placed in plasma baths in vitro undergo volumetric outward expansion. Furthermore, addition of the stainless steel outer ring does not direct the casein portion of the ARC to centripetally constrict the luminal area. Future in vivo studies would be necessary to evaluate activities of ARCs placed in dogs with and without outer stainless steel rings in place.

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b. Scientific Products-American Hospital Supply Corp, McGaw Park, Ill.
c. AE50, Mettler-Toledo Inc, Columbus, Ohio.
d. DSC-S75, Sony Corp, Tokyo, Japan.
e. Digimatic caliper, model No. CD-6"CS, Mitutoyo Corp, Aurora, Ill.
f. Image J, version 1.37, National Institute of Health, Bethesda, Md. Available at: rsbweb.nih.gov/ij. Accessed September 1, 2005. JMP, version 6.0.0, SAS Institute Inc, Cary, NC.

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