Supplemental Materials

Magnesium-based bioresorbable flow diverter for intracranial aneurysms: a pilot study of biocompatibility and bioresorption in a rabbit vascular model

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Supplemental Methods

MgBRFD implantation and harvesting

Rabbits were anesthetized using intravenous sodium thiopental (20 mg/kg) as an induction agent and maintained with 1%–2% isoflurane inhalation anesthesia. Local xylocaine was infiltrated at the skin incision site before sheath insertion. The femoral artery was exposed using the cutdown technique. A 4 F sheath was inserted and exchanged for a 5 F sheath before guiding a 0.014-inch wire to the thoracic aorta. Then, a MgBRFD was implanted through a 5 F catheter into the abdominal aorta. Angioplasty and OCT were performed via the same catheter.

Euthanasia was performed with an overdose of sodium thiopental (100 mg/kg) while under 1%–2% isoflurane inhalation anesthesia. Perfusion fixation and specimen harvesting were then performed as described in a previous study[11].

μCT analysis

Scans were acquired using a source voltage of 80 kV and source current of 100 μA through no filter. Projection images were taken every 0.2° over a 360° range with an exposure time of 0.8 seconds. Images were reconstructed and displayed using manufacturer-provided software (CTvox version 3.3.1; Bruker-MicroCT) to produce image data with a voxel resolution of 6 μm. Thresholding was based on the diameter of the magnesium wire prior to implantation.

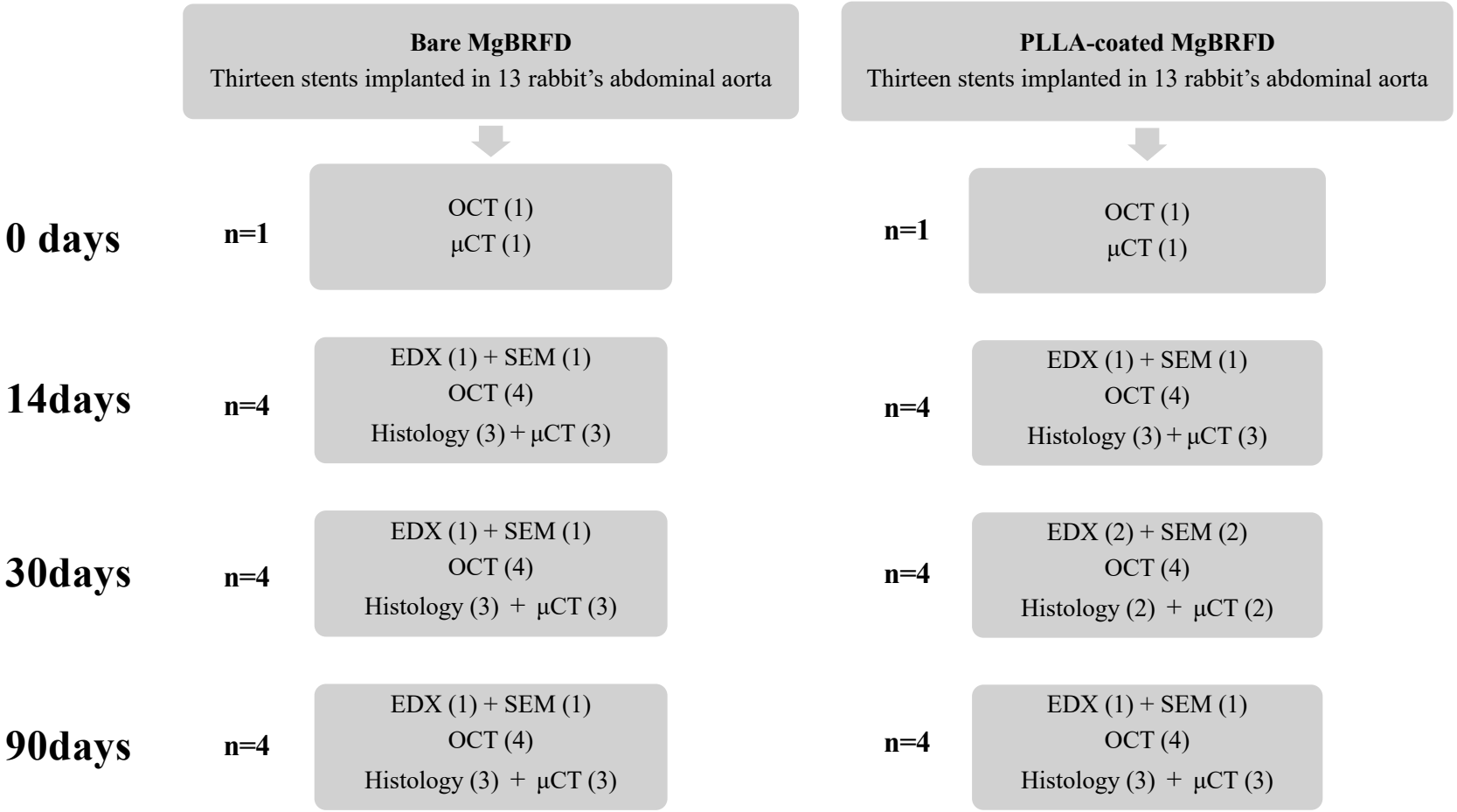
SEM and EDX analysis

Tissue processing for evaluation of stent surface endothelialization by SEM was performed in the same manner as described in a previous study[11]. Tissue prefixation for EDX was performed similarly to SEM, creating a central cross-section of the vessel in which the FD was implanted. After post-fixation with 2% osmium tetroxide in distilled water at 4° C for 2 hours, the samples were dehydrated in a graded ethanol series(50%, 60%, 70%, 80%, 90%, 95%, 99%, and 100%) and treated with 100% propylene oxide followed by Epon 812. Specimens were prepared by embedding them in Epon 812 and cutting the cross-sections with a diamond knife. The samples were observed with a JSM-7900 scanning electron microscope (JEOL, Tokyo, Japan).

Histopathological processing and analysis

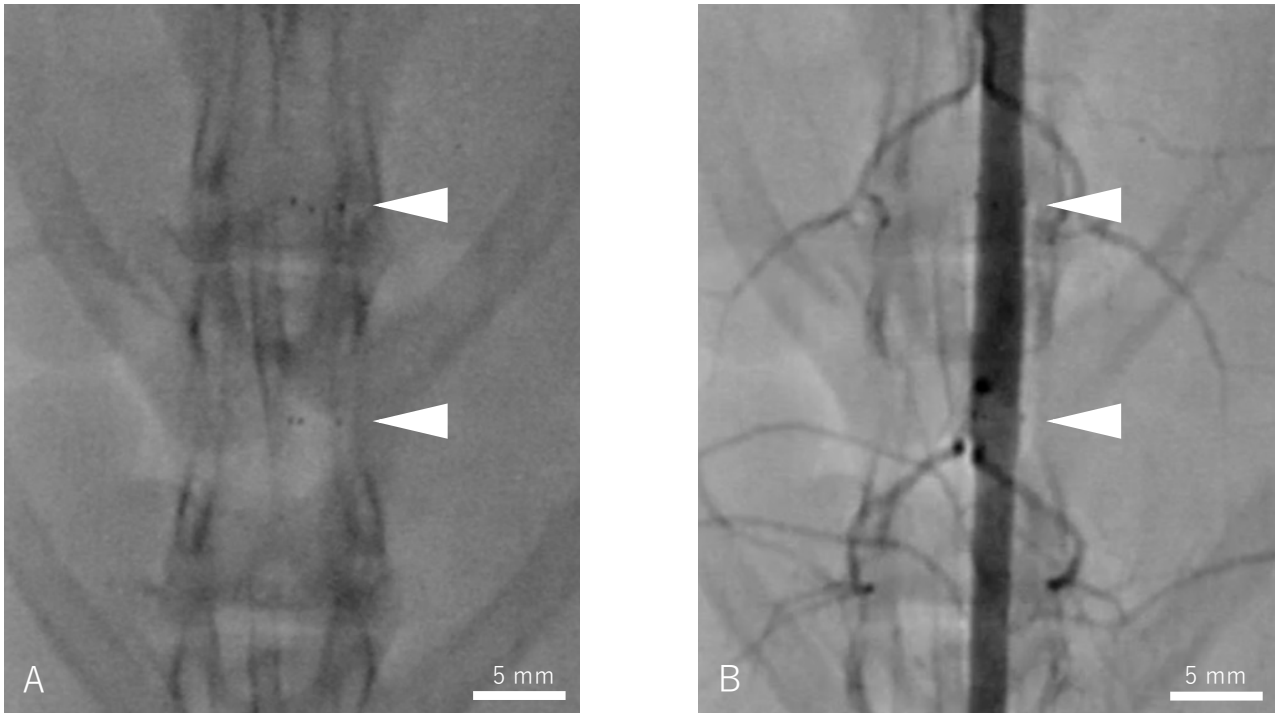
Histopathological processing was performed as described in a previous study[11]. Histopathological specimen preparation and hematoxylin and eosin staining, special staining, and immunohistochemical staining were outsourced to a specialized company (Biopathology Institute Co., Ltd, Oita, Japan). For

immunohistochemistry staining, the slides were blocked with skim milk and incubated with primary antibodies overnight at 4° C. Expression of the target molecules was visualized using 3,3'-diaminobenzidine (DAB) solution and nuclei were stained with hematoxylin. The primary antibodies were α-SMA polyclonal antibody, n-term (1:100; GeneTex, GTX89701), goat Iba-1 polyclonal antibody (1:100; Abcam, ab5076), and mouse Ki67 monoclonal antibody (1:100; Novus, NBP2-22112). Histopathological images were obtained using a Slideview VS200 Scanner (Olympus, Tokyo, Japan). Cell counts and neointima thickness were analyzed using the viewing software OlyVIA 4.1 (Olympus) and the BZ-X 800 analyzer (Keyence, Osaka, Japan).



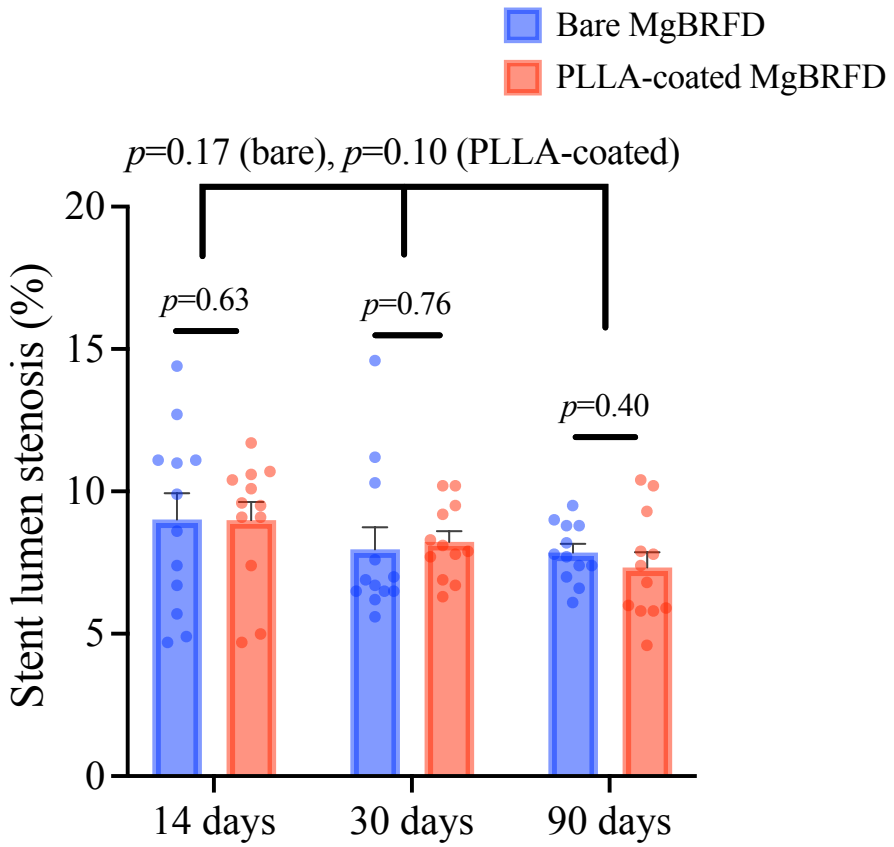
Supplemental Figure 1. study flow chart

Samples assigned for SEM and EDX analysis could not be used for histopathological analysis because the entire sample was required for SEM and EDX analysis. μCT could not perform samples assigned for SEM and EDX analysis due to the different tissue processing required. Due to an unexpected tissue processing failure on one sample of the PLLA-coated MgBRFD at 30 days, another sample was performed for EDX and SEM analysis.



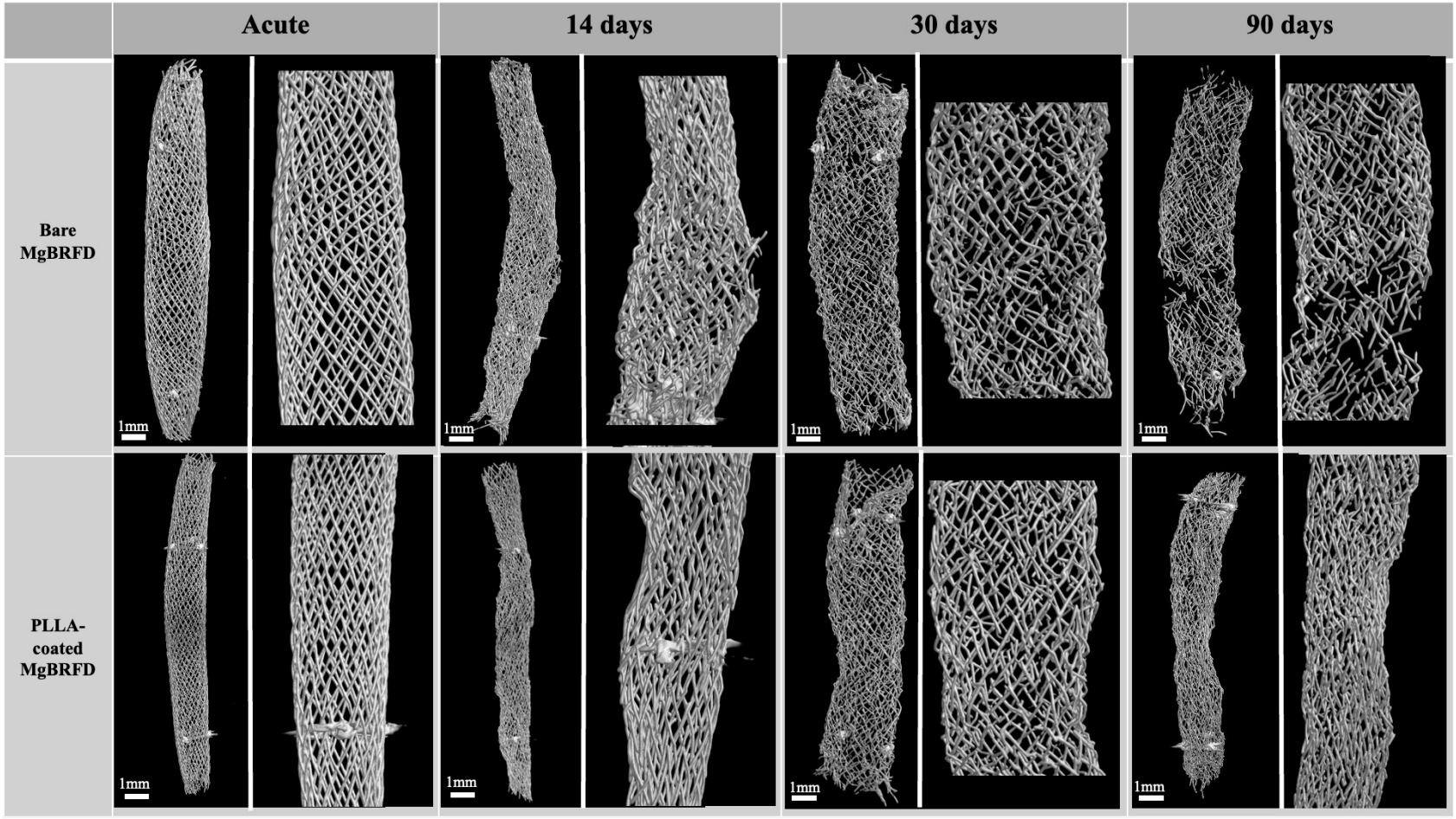
Supplemental Figure 2. Angiography 3 months after PLLA-coated MgBRFD implantation
A is a fluoroscopic image and **B** is an angiographic image. **A** shows that the stent is not radiopaque except for the radiopaque markers at both ends. **B** shows the patency of the aorta and its side branches covered by the stent. The white arrowheads indicate the radiopaque markers.

Follow-up	Device	Lumen area stenosis (%)
14 days	Bare	9.5 ± 0.8
	Coated	9.0 ± 0.6
	p-value	0.73
30 days	Bare	8.0 ± 0.8
	Coated	8.2 ± 0.4
	p-value	0.18
90 days	Bare	7.9 ± 0.3
	Coated	7.3 ± 0.5
	p-value	0.31



Supplemental Figure 3. Lumen area stenosis rate confirmed by OCT

There were no significant differences between stents at any time point and no significant changes over time for each stent. Analyses were performed using unpaired 2-tailed t test and 1-way ANOVA. Data are means ± standard error.



Supplemental Figure 4. μCT results over time for MgBRFDs

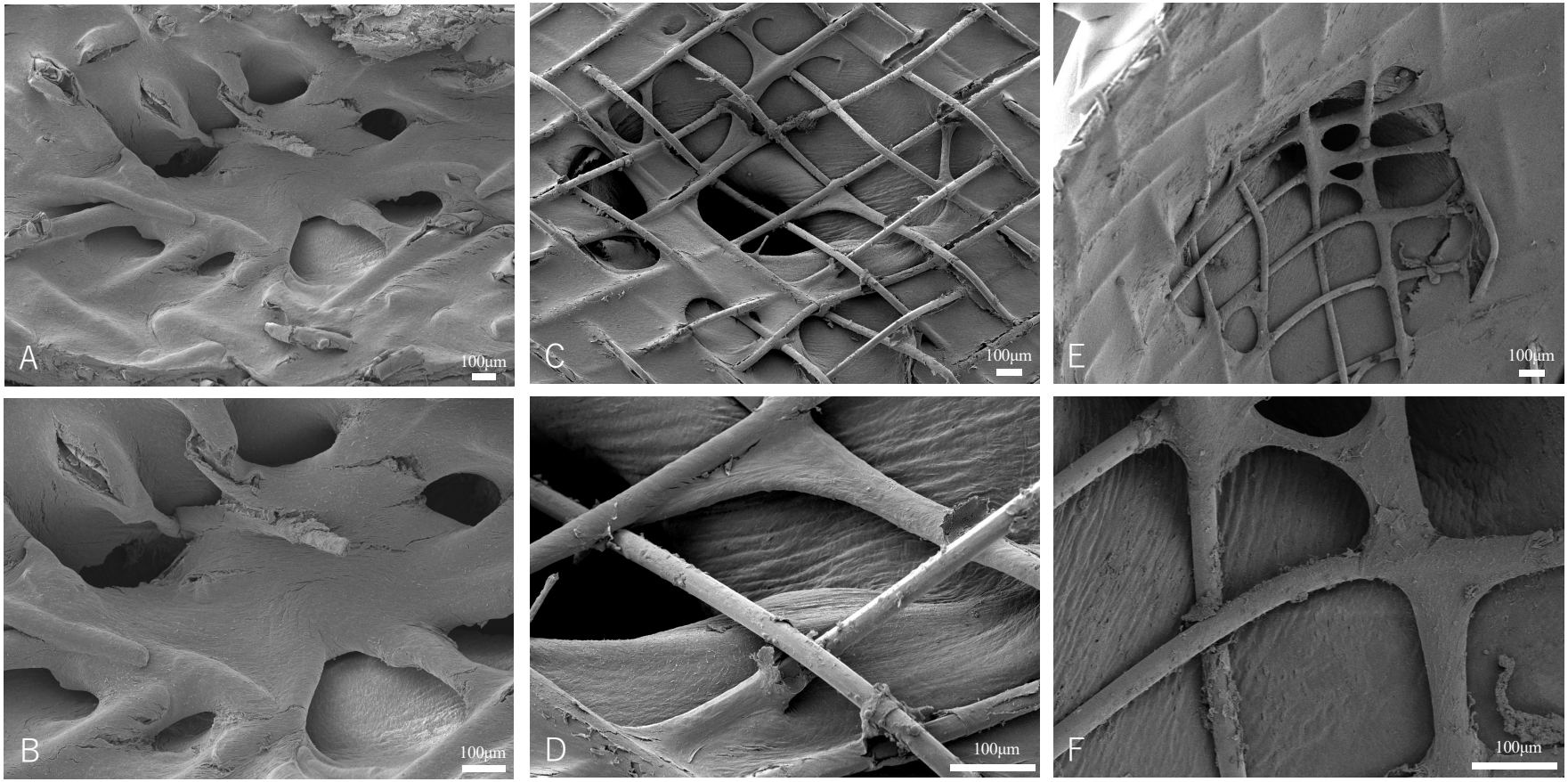
In each MgBRFD at each follow-up time point, the left side of the image is the whole image and the right side is a magnified image. At 14 days, bare MgBRFD showed the destruction of the stent structure over the side branch in the magnified image. At 30 days, bare MgBRFD showed more stent strut fractures in magnified images than PLLA-coated MgBRFD. At 90 days, bare MgBRFD showed areas of large stent structure loss.

	Control		14 days		30 days		90 days	
at.%	PLLA-coated	Bare	PLLA-coated	Bare	PLLA-coated	Bare	PLLA-coated	Bare
O	6.0±2.1	2.3±0.8	27.5±18.3	53.8±4.7	53.1±14.8	56.9±3.2	49.0±7.6	58.8±5.9
Mg	90.4±2.0	91.8±4.8	61.9±28.4	0.7±0.2	0.1±0.05	0.1±0.02	0.2±0.03	0.4±0.1
Al	0.3±0.04	0.3±0.05	0.4±0.2	0.6±0.1	0.4±0.1	0.5±0.1	0.3±0.04	0.3±0.1
P	0±0	0±0	4.3±7.0	23.6±2.2	26.1±7.5	20.8±1.6	24.2±3.4	19.8±2.7
Ca	0±0	0±0	1.3±2.0	10.9±2.2	8.81±3.6	11.9±1.9	16.7±3.7	13.1±2.4
Zn	1.0±0.1	1.4±0.8	1.7±1.2	2.8±0.8	2.0±1.3	1.8±0.3	1.1±1.9	0.7±0.3
Y	2.1±0.3	3.6±3.3	3.6±2.3	7.3±1.3	9.0±3.4	7.5±0.9	7.9±1.9	6.6±1.1
Yb	0.2±0.02	0.7±0.9	0.3±0.2	0.4±0.1	0.4±0.1	0.4±0.1	0.4±0.1	0.4±0.1

Supplemental Table 1. Point analysis results of the degradation products on the cross section of the MgBRFDs over time by EDX
Data are means ± standard deviation. The point analysis showed the same trend as the mapping analysis. Up to 14 days, More Mg elements remained in the PLLA-coated MgBRFD, but after 30 days, no difference was observed between the two MgBRFDs. The decomposition product of the Mg alloy is presumed to be calcium phosphate.

	14 days			30 days			90 days		
	Bare	PLLA-coated	p-value	Bare	PLLA-coated	p-value	Bare	PLLA-coated	p-value
Neointimal thickness (μm)	156 ± 5.0	180 ± 6.6	0.11	151 ± 7.1	179 ± 5.3	0.12	155 ± 5.7	197 ± 7.4	0.003
Iba-1 positive cells	220 ± 24.2	202 ± 29.5	0.64	168 ± 23.3	167 ± 29.1	0.97	136 ± 17.1	84 ± 13.0	0.03
Ki67 positive cells	193 ± 16.8	265 ± 31.6	0.06	84 ± 11.3	101 ± 11.5	0.33	19 ± 4.0	50 ± 8.5	0.005
% struts with Giant cell (%)	77 ± 2.5	69 ± 4.3	0.12	74 ± 3.6	71 ± 2.5	0.59	46 ± 5.0	60 ± 3.0	0.03

Supplemental Table 2. Histopathological Outcomes
Analyses were performed using unpaired 2-tailed t test. Data are presented as means ± standard error.



Supplemental Figure 5. Neointimal coverage of stent struts placed in the abdominal aorta over side branches (lumber arteries) on scanning electron microscopy
A and **B**) Neointimal coverage 14 days after implantation of low and high magnification PLLA-coated MgBRFDs, respectively. **C** and **D** show the neointimal coverage over the side branches 6 months after implantation of low and high magnification CoCr-FDs, respectively. **E** and **F** show the neointimal coverage over the side branches 24 months after implantation of low and high magnification CoCr-FDs, respectively. Compared to CoCr-FD, excellent intimal coverage is observed early, even over side branches where neointimal coverage is difficult to achieve with MgBRFD.