Regeneration and Reinforcement of the Aorta Using Scaffolds Composed of Biodegradable Non-Woven Fabric in a Dog Experiment: A Preliminary Report for a Rapid Communication

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To regenerate the aorta we devised a new vascular scaffold with multilayered tube structure. The innermost layer was a fabric tube made of poly (lactide/caprolactone) produced by electro-spinning method. Fabric roles of poly (lactide/caprolactone) and/or poly (lactide) produced by melt-blow or knit method were overlying the innermost layer. An outer layer of anastomotic site was covered with a role of poly (glycolic acid) fabric produced by melt-blow method. The multilayered vascular scaffold was implanted in the native abdominal aorta of dog. Chronological observation of tissue regeneration and graft degradation were also conducted in rats and dogs for detail analyses. The aorta was regenerated in 10 months in almost similar structure to the native aorta. In the early phase after implantation, the outer polymer layers, initially polymer fabrics themselves, were replaced by self-organizing membranes. The outer layers were considered to work for reinforcement of the innermost layer. The fabrics of innermost tube maintained the original structure and degraded slowly. In conclusion the new vascular scaffold can regenerate the canine aorta with almost similar structure to the native aorta.

Key words: regeneration of aorta, dog experiment, biodegradable polymer, multi-layered scaffold

1. Introduction

Successful regeneration of the aorta has not been reported, and how to regenerate the aorta using biodegradable scaffold is not known because there is a dilemma between the regeneration of the aorta and the biodegradable scaffold: Although the regenerated aorta should be strong enough to withstand aortic blood pressure, the aortic wall is not strong enough during the regeneration until maturation is completed after the regeneration. On the other hand, the scaffold for the regeneration of the aorta becomes weak before the regeneration because the scaffold should be degraded and absorbed in order to change into the regenerated aorta and the degradation and absorption of the scaffold involves its weakening. Thus, the maintenance of strength of the scaffold makes the regeneration of a strong aorta impossible; on the other hand, the regeneration of a strong aorta requires the weakening of the scaffold.

To overcome this dilemma, we devised a new vascular scaffold with multilayered tube structure. In

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the early phase (within several weeks) after implantation, many interstitial cells infiltrate into the outer layers and form a dense granulation tissue, which works as the reinforcement against the aortic blood pressure. Thus, the outer layers turn into reinforcement through infiltration of interstitial cells (self-organizing reinforcement) within several weeks. The innermost tube has narrow spaces between the fibers, and the degradation and cell infiltration begin slowly after the outer layers form the self-organizing reinforcements completely. During this period of time in which the tube is slowly degraded, slow regeneration of the media of the aorta occurs on and in the luminal side of the tube.

In this preliminary report, we present (A) successful regeneration of the aorta in a large animal model and (B) the characteristics in tissue regeneration and degradation of four kinds of fabric used in the scaffold to regenerate the aorta.

2. Materials and Methods

2.1 *Non-woven fabric for scaffolds*

We prepared four kinds of non-woven fabric, namely, ESD(LA/CL), MB(LA/CL), MB(PGA) and MB(PLA). ESD(LA/CL) is made by electro-spinning deposition (ESD)[1)] and composed of poly(75-50% lactide/25-50% caprolactone). ESD(LA/CL) has small spaces between the fibers, and the mean size of the spaces is less than 2 μm.

MB(LA/CL) and MB(PGA) are made by the melt-blow method[2)] and are composed of poly(75% lactide/25% caprolactone) and poly(glycolic acid), respectively. They have relatively large spaces between the fibers, the median sizes of which are more than 40 μm. MB(PLA) was a knit composed of poly(lactide) and the mesh-size is 100-300μm. These fabrics were sterilized with ethylene oxide before use.

2.2 *Animal experiments*

All animal care, housing, and surgical and anesthetic procedures were performed in accordance with the Animal Care Guidelines of the Committee for Animal Research of Doshisha University, Nara Medical College, and Kyoto University.

2.2.1 *Experiment 1*

In Experiment 1, rats were used as the experimental animals. Female Wister rats weighing 150 g, purchased from Shimizu Laboratory Animal Supply Co., Ltd. (Kyoto, Japan), were used in this study. All rats were maintained under standard conditions (a light-dark cycle of 12:12 h, mean temperature of 23 degrees Celsius, and mean humidity of 50%) with free access to water and feeding with standard pellets. Before the study, the rats were housed in the laboratory for one week.

All surgical procedures were performed by one surgeon under sterile conditions. Under general anesthesia with inhalation of isoflurane and the intraperitoneal injection of pentobarbital (Somnopentyl®; Kyoritsu Seiyaku, Tokyo, Japan) at 30 mg/kg of body weight, the rats were fixed in the abdominal position. The hair on the back was shaven. The skin was cleaned with a solution of 5% chlorhexidine plus 80% ethanol, and was sterilized with 10% povidone iodine solution. Then, the rats received a 10-mm-long skin incision on the back, and subcutaneous tissue was dissected bluntly to make a “skin pocket” in the subcutaneous space of the back. One of four kinds of scaffold was set in the pocket individually, and then the skin pocket was closed with interrupted skin sutures using 5-0 nylon monofilament thread.

(Microscopic Evaluation)

The rats were sacrificed on day 14 and 21 after surgery by intraperitoneal injection of pentobarbital at 100 mg/kg of body weight. An U-shaped skin incision was performed around the skin pocket and the skin pocket containing the scaffold was removed *en bloc* surgically as the tissue specimen to be examined for the status of cell infiltration microscopically. The removed tissue specimens were fixed in 10wt% neutral formalin solution, and were processed using the standard

procedure into histological specimens of thin sections (4μm) stained by the Hematoxylin-Eosin (H-E) method.

2.2.2 *Experiments 2, 3 and 4*

In Experiments 2, 3 and 4, dogs were used as experimental animals. Non-pregnant female Beagle dogs aged one year and weighing 10 kg were purchased from Shimizu Laboratory Animal Supply Co., Ltd. During the experimental period, all dogs were housed separately and maintained under the set conditions for more than one week before the study. Standard laboratory dog chow and water were freely available.

All surgical procedures were performed under sterile conditions by a team of surgeons. As basic anesthesia, the dogs were given intravenous anesthesia in the form of sodium pentobarbital at a dose of 34 mg/kg of body weight. Then, the dogs received tracheal intubation and were controlled by inhalation anesthesia with 40% O_2 + sevoflurane or isoflurane during the surgery. Under the above-described general anesthesia, the dogs were fixed in the dorsal position. The hair on the abdomen was cut and shaven. The skin was cleaned with a solution of 5% chlorhexidine plus 80% ethanol, and it was sterilized with 10% povidone iodine solution.

After the surgery, two kinds of antibiotic were given for 3 to 7 days according to the postoperative conditions of the surgical wound.

(Experiment 2)

A 15-cm-long laparotomy incision was made along the midline of the abdomen of the dogs. The peritoneum on the common iliac artery was cut and the iliac artery was exposed. After removing connective tissues around the artery, MB(PGA), MB(PLA) or MB(LA/CL) was tightly wrapped around the artery. Then, the cut ends of the peritoneum were sewn together, and the laparotomy incision was closed by 2-layered sutures.

(Experiment 3)

In a similar manner to Experiment 2, laparotomy was performed and the peritoneum on the aorta was cut. The abdominal aorta, from the furcations of the renal artery to the common iliac artery, was exposed and separated from the surrounding tissues. During this separation, the lumbar arteries were divided under ligations. After an intravenous injection of low-molecular-weight heparin at 2,000 U, two pairs of forceps were set to obstruct the aorta and the aortic wall was cut up to half of the circumference between two forceps. The lumen of the aorta was rinsed with heparinized saline, and the cut ends of the aortic walls were sown together by three interrupted sutures with 6-0 polypropylene monofilament thread. MB(PGA) was wrapped tightly in triplicate around the aortic wall over the suturing line to reinforce the sutured wall, and then compressed with the fingers for 5 minutes for hemostasis. Then, the cut ends of the peritoneum were sewn together, and the laparotomy incision was closed by 2-layered sutures.

After surgery, anti-coagulant therapy was administered with low-molecular-weight heparin at 2,000 U, aspirin at 100 mg, and/or warfarin at 1 mg per day.

(Experiment 4)

The same as in Experiment 3, the aorta was separated from surrounding tissues and two pairs of forceps were set. Then, the aorta was divided completely along the circumference between two forceps. After rinsing with heparinized saline, a tube of ESD(LA/CL) of 4 cm in length and 8 mm in diameter (the innermost tube) was interposed between the two cut ends of the aorta, and the aorta and the tube were anastomosed in an end-to-end manner: the cut ends of the tube wall and the aortic wall were sown together with 12 sutures of 6-0 polypropylene monofilament threads. Over the tube and both anastomotic portions, MB(LA/CL) and/or MB(PLA) of 6 cm in width and 5-15 cm in length were wrapped tightly to reinforce the tube. Then, each anastomotic portion was reinforced by MB(PGA) with a width of 3 cm wrapped twice around the portion. Then, the cut ends of the peritoneum were

sewn together, and the laparotomy incision was closed by 2-layered sutures. After surgery, anti-coagulant therapy was administered with low-molecular-weight heparin at 2,000 U, aspirin at 100 mg, and/or warfarin at 1 mg per day.

(Macroscopic and Microscopic Evaluations)

The dogs were sacrificed 4 weeks (in Experiments 2 and 3) and 10 months (in Experiment 4) after surgery by intravenous injection of pentobarbital at 100 mg/kg of body weight. A re-laparotomy was performed and the implanted scaffolds were removed *en bloc* with the surrounding tissues containing the native arterial wall as the tissue specimens for macroscopic and microscopic examinations.

After macroscopic examinations, the removed tissue specimens were fixed in 10wt% neutral formalin solution and processed using the standard procedures into microscopic specimens of thin sections (4 μm) stained by the H-E method, Elastica-van Gieson method and Orcein method.

We examined the specimens microscopically for cell infiltration into the scaffolds as self-organized reinforcement in Experiments 2 and 3, for the status of the reconstruction and repair of the cut wound of the aortic wall in Experiment 3, and for the effects of the scaffold on regeneration of the aorta in Experiment 4.

3. Results

3.1 *Experiment 1*

The results are shown in Fig. 1. There are few cells infiltrating in the inner side of ESD(LA/CL) even on day 21 (Fig. 1-A), whereas numerous cells are infiltrating on day 14 in the full thickness of MB(LA/CL) (Fig. 1-B), MB(PLA) and MB(PGA) (Fig. 1-C).

3.2 *Experiment 2*

Fig. 2 shows the results, with numerous interstitial cells infiltrating into the full thickness of both of MB(LA/CL) (Fig. 2-A), MB(PLA) and MB(PGA) (Fig. 2-B), which looked to be the self-organizing reinforcements around the arterial walls.

3.3 *Experiment 3*

MB(PGA) wrapped around the aortic wall cut up to half of its circumference stopped the bleeding well via resisting the blood pressure. The cut wound of the aortic wall was repaired well: the intima and the media were regenerated, the cut wound became flat, and no aneurysm was found (Figs. 3A and 3-B). Around the cut wound, there was a layer of MB(PGA) with infiltrating interstitial cells (Figs. 3-C and 3-D).

3.4 *Experiment 4*

In dogs sacrificed 10 months later, the aorta was regenerated macroscopically and microscopically. Macroscopically, the aorta was regenerated into a shape similar to that of the native aorta. Macroscopically, the regenerated aorta serves the function of the aorta: it has the aortic lumen through which the aortic blood flows and where there is no thrombus. Microscopically, the regenerated aortic wall was composed of the intima, the media, and the adventitia (Fig. 4-A), which looked very similar to the native ones (Fig. 4-B). In particular in Elastica-van Gieson stain (Figs. 4-A② and 4-A③) and Orcein stain, the layer of the regenerated media was rich in elastic fibers and smooth muscle cells, similarly to the native medial layer.

4. Discussion and Conclusion

The results in Experiment 1 show that few cells infiltrate into ESD(LA/CL), whereas numerous cells infiltrate into all of MB(LA/CL), MB(PLA) and MB(PGA). The reasons for this are thought to be that fabric made by the common ESD method has narrow spaces between the fibers where cells hardly infiltrate, and that the melt-blow method or knitting makes fabric with wider inter-fiber spaces where cells and blood vessels easily infiltrate.

In Experiment 2, the three kinds of fabric made by the melt-blow method or knitting were tested in terms of whether or not the fabric acts as a scaffold that

forms reinforcement through the infiltration of interstitial cells into the scaffold (self-organizing reinforcement). The results indicate that three types of fabric can act well as self-organizing reinforcement, and that these three kinds of fabric made by the melt-blow method or knitting, when wrapped around the outer side of the aortic wall, will be useful to reinforce this wall.

In Experiment 3, MB(PGA) was tested for reinforcing effects against the arterial blood pressure acting on the cut end sewn by thread. When the arterial wall was cut up to half of the circumference, three interrupted sutures sewing the cut ends together could never control the massive bleeding via resisting the blood pressure. However, the fabric of MB(PGA) wrapped around the aortic wall over the cut wound stopped the bleeding well via resisting the blood pressure. The results in 4 weeks later indicate that MB(PGA) reinforced the cut wound of the aortic wall via resisting the blood pressure after surgery for 4 weeks and allowed the cut wound to be repaired well.

Experiment 4 revealed that the aorta in the shape of a tube was regenerated almost completely. MB(LA/CL), MB(PLA) and MB(PGA) became self-organizing reinforcements within several weeks and reinforced the innermost tube of ESD(LA/CL) against the aortic blood pressure, while ESD(LA/CL) formed the aortic media, the regeneration of which needed a much longer period of time than the formation of the self-organizing reinforcement.

In conclusion, the aorta in the whole tube shape was regenerated in a dog experiment using a scaffold composed of an innermost tube of ESD(LA/CL) and outer reinforcing layers of MB(LA/CL), MB(PLA) and MB(PGA).

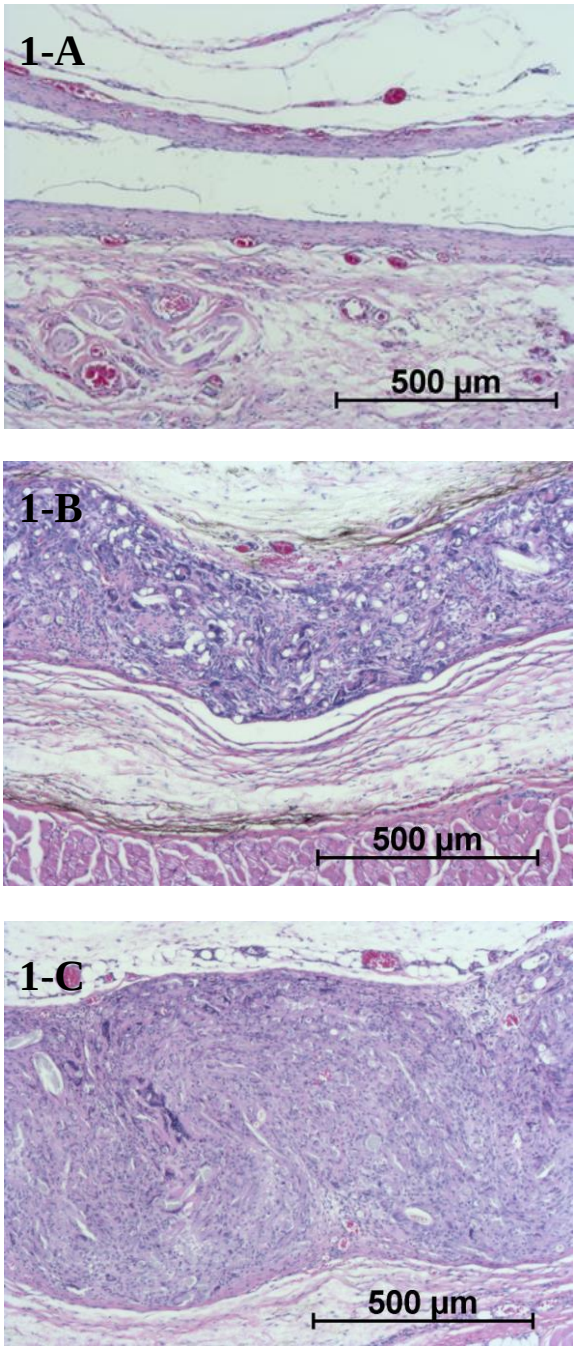
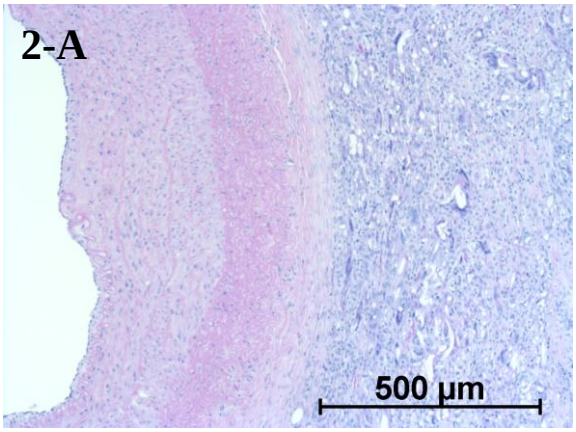
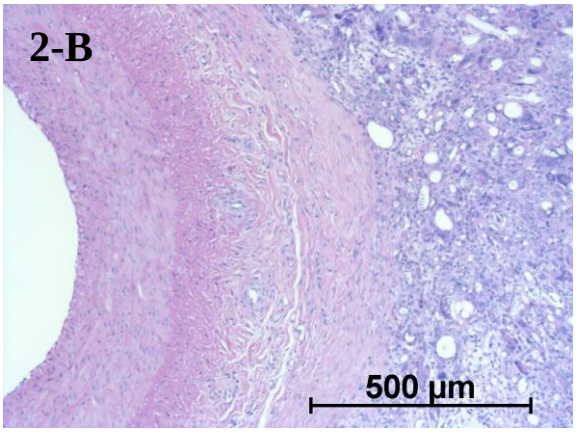


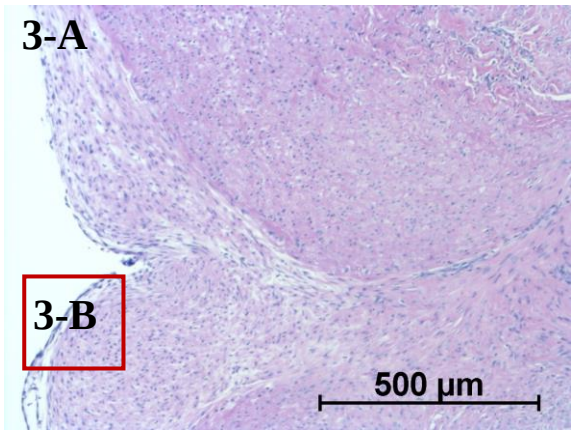
Fig. 1. The fabrics after implantation in rats.
Fig. 1-A: ESD(LA/CL) taken 21 days after implantation. Few cells are infiltrating in the fabric.
Figs. 1-B and 1-C: taken 14 days after implantation. Numerous cells are infiltrating on day 14 in the full thickness of MB(LA/CL) and MB(PGA), respectively.

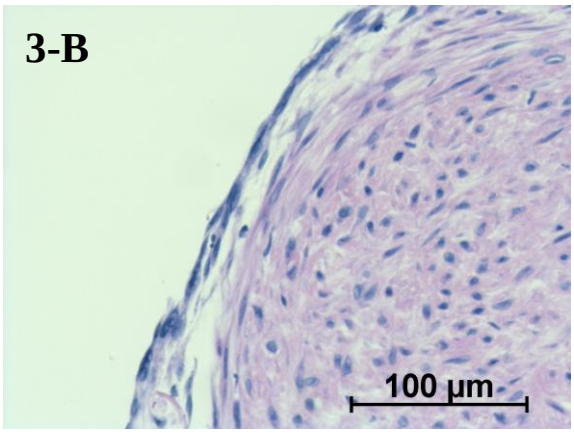


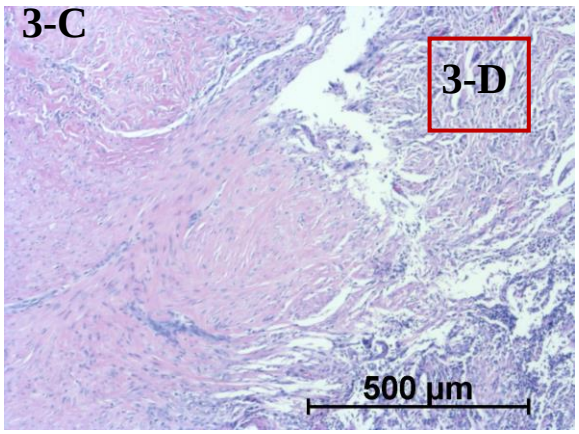


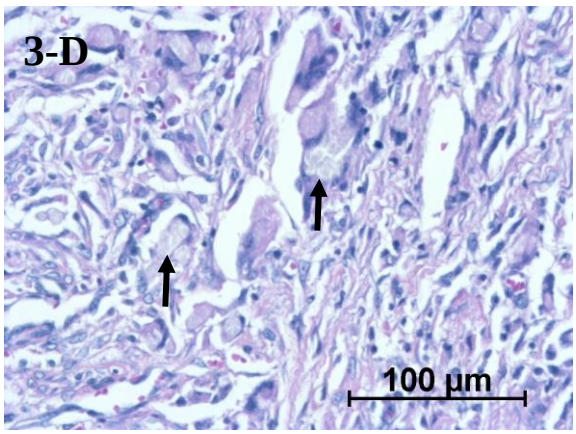
Figs. 2-A and 2-B: The fabrics implanted around the artery in dog.

Four weeks after wrapping around the artery, numerous interstitial cells are infiltrating into the full thickness of both of MB(LA/CL) (Fig. 2-A) and MB(PGA) (Fig. 2-B), which look to be the self-organizing reinforcements of the artery.



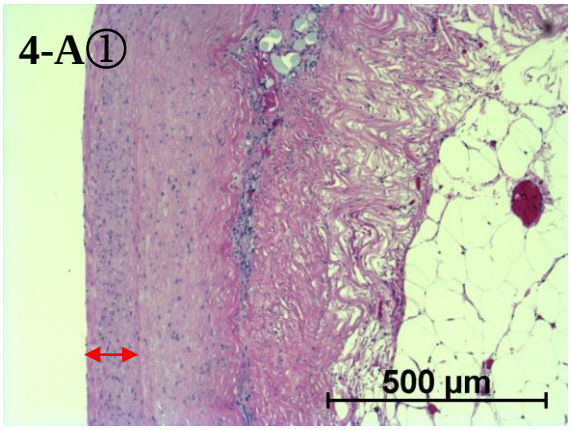


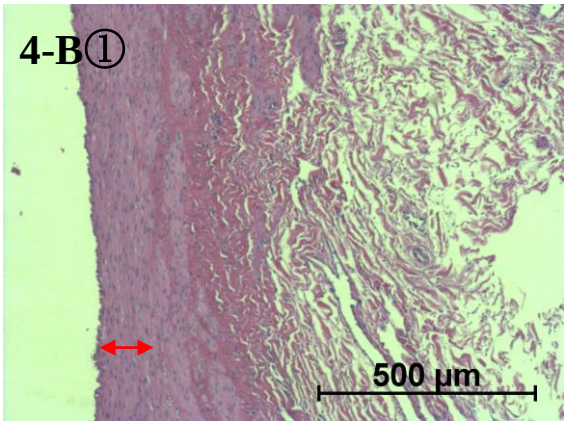


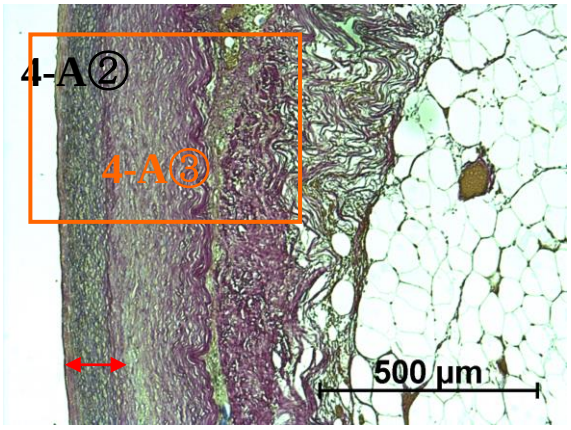


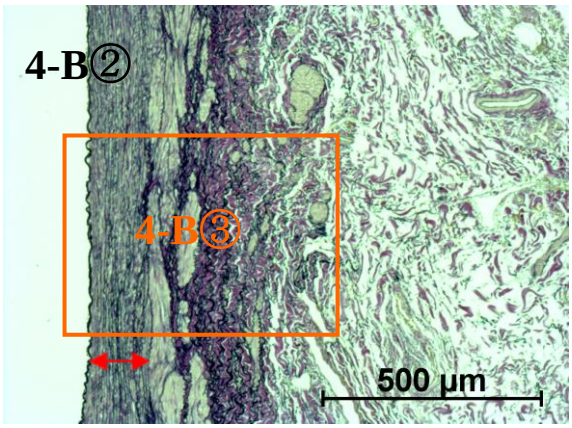
Figs. 3-A, 3-B, 3-C and 3-D: The repair of the arterial wall reinforced by MB(PGA).

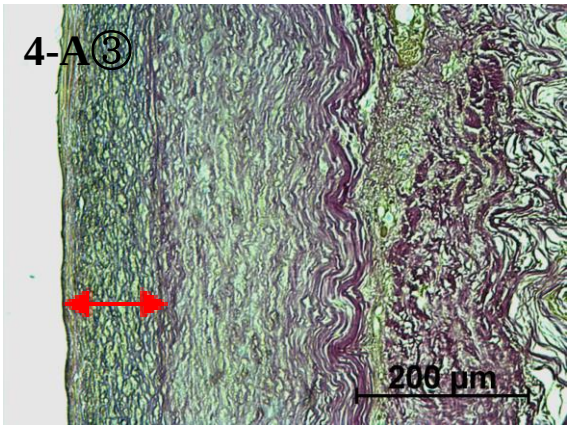
The cut wound of the aortic wall (Figs. 3-A and 3-B) 4 weeks after cutting up to half of the circumference of the aortic wall. The intima and the media are regenerated, the cut wound is flat, and no aneurysm is found. The outside of the cut wound of the aortic wall (Figs. 3-C and 3-D). There is a layer of MB(PGA) with infiltrating interstitial cells. Arrows indicates the remaining MB(PGA) fibers.

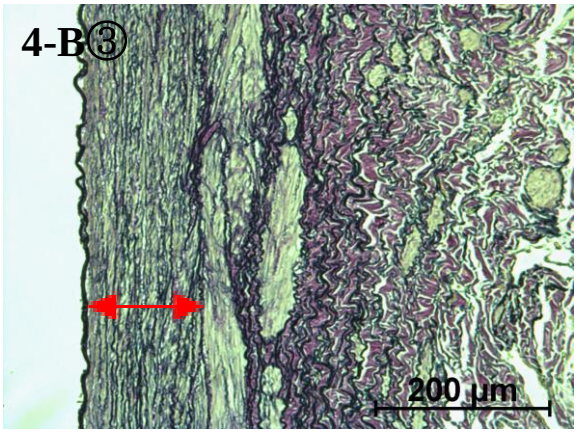












Figs. 4-A and 4-B: Regenerated aortic wall (Fig. 4-A) and native aortic wall (Fig. 4-B).

Figs.4-A①and B① : H-E stain

Figs.4-A②,③,4-B②, and ③: Elastica-van Gieson stain

The medial layer (red arrow ⟷) in the regenerated aorta is similarly regenerated to that in the native aorta.

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