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We developed a novel large-diameter graft “Triplex®” that uses a non-biodegradable material as a coating material. This time, in order to demonstrate the physical properties of Triplex® grafts, we conducted physical tests in accordance with the international guidelines, using the collagen coated vascular grafts (Hemashield, Boston Scientific, Natick, Massachusetts, USA) as the controls. The grafts were tested with regard to strength (burst strength, circumferential tensile strength, longitudinal tensile strength), suture retention strength, integral water permeability, water leakage (needle puncture, after using clamp), and change in luminal diameter following pacing stress according to ISO7198 and FDA guidance. As indicated by the results, we experimentally demonstrated that uniquely designed vascular graft Triplex® led to less blood leakage from the vascular graft and less leakage from the needle puncture, although it has fundamental physical properties comparable to those of the vascular grafts using biodegradable material that has been utilized conventionally in clinical settings. Triplex® is expected to play its role as a clinically beneficial next-generation vascular graft.

Keywords: vascular graft, sealed graft, porosity, basic research, physical testing

INTRODUCTION

Recent years, vascular diseases including aortic aneurysm and dissection or arteriosclerosis obliterans are increasing, especially in the thoracic region, polyester grafts with coating of bioabsorbable material are introduced typically. However, complications including pyrexia, increased C-reactive protein (CRP), and increased white cell count have been reported for shield vascular grafts that might be associated with the bioabsorbable coating material such as gelatin and collagen.1-6) In terms of the fabrication process of the polyester grafts, they are made by the weaving technique or knitting technique. The woven grafts have several problems including hardness, easiness to ravel, and frequent bleeding from needle puncture. The knitted grafts are softer than the woven grafts, and thus have advantages such as good operability at surgical procedure, cells’ easiness to go into the meshes of the knitted fabric due to low fiber density, and high affinity with the body tissue, although some indicated the dilation of the knitted grafts following surgical procedure over time.7-12)

Aiming to solve these problems, we developed Triplex®, a vascular graft having a unique three-layered structure without using absorbable materials. To facilitate cell infiltration and enhance their connectivity with tissues, Triplex® grafts employ a knitted porous matrix for the inner and outer layers, and a nonporous sheet made of a non-biodegradable material, elastomer, for the middle layer. In addition, for prevention of postoperative dilation over time, the outer layer is dilated in advance (predilation process). With its composing materials and unique structural design, Triplex® may be used instantly in urgent occasions and leads to less bleeding from a needle puncture or the body of the graft. Also,
Burst strength

This test was conducted in accordance with ISO 7198:1998 (E) 8.3.3.2 “Determination of probe burst strength.” The samples were cut to pieces in length of 2 cm, and each piece was cut open in a longitudinal direction to make a sheet-like test piece. Each test piece was fixed on the fixture having 8-mm hole so that the piece covered the hole completely. After the fixture with the test piece was mounted on the tensile testing machine (Strograph-T, Toyo Seiki Seisaku-sho, Ltd., Tokyo, Japan), a 6-mm diameter round bar was pushed from the inside into the outside at a rate of 125 mm/min and destructed the test piece. The maximum load (N) was recorded as the burst strength.

Circumferential tensile strength

This test was conducted in accordance with ISO 7198 8.3.1 “Determination of circumferential tensile strength.” Each test graft was cut into pieces of a length equal to or more than free internal diameter. The values measured using a stainless steel straight ruler (S-100, Lion Office Products Corp., Tokyo, Japan) were recorded [L (mm)].

Using a fixture designated by ISO 7198, each test piece was pulled to the vertical direction at a rate of 125 mm/min. on a tensile testing machine (Strograph-T, Toyo Seiki Seisaku-sho, Ltd., Japan), and the maximum load \([T_{\text{max}} (N)]\) was recorded.

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\text{Circumferential tensile strength} = \frac{\text{Maximum load}}{\text{Length}} = \frac{T_{\text{max}}}{2L} \text{ (N/mm)}
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Longitudinal tensile strength

This test was conducted in accordance with ISO 7198 8.3.2 “Determination of longitudinal tensile strength.”

Each vascular graft was cut into 50- mm length, and the both ends of the piece were fixed on the tensile testing machine (Strograph-T, Toyo Seiki Seisaku-sho, Ltd., Japan). The piece was pulled in a longitudinal direction at a rate of 125 mm/min, and the maximum load (N) was recorded.

Suture retention strength

This test was conducted in accordance with ISO 7198 8.8 “Determination of suture retention strength.”

Straight-across procedure: Each vascular graft was cut vertically to the axis, and at the point 2 mm away from the cut surface, a stainless wire (3–0 stainless wire, BEAR Medic Corporation, Ibaraki, Japan) was pushed...
through the graft piece. The vascular graft and the stainless wire were fixed on a tensile testing machine (Strograph-T, Toyo Seiki Seisaku-sho, Ltd., Japan), and pulled at a head speed of 200 mm/min at three points of the graft piece.

**Oblique procedure:** Each vascular graft was cut at an angle of 45 degrees to the axis, and at the point 2 mm away from the cut surface, a stainless wire was pushed through the graft piece. The vascular graft and the stainless wire were fixed on a tensile testing machine and pulled with the same procedure as the straight-across procedure at three points of the graft piece – toe, 90°, and heel positions.

**Integral water permeability/leakage**

This test was conducted in accordance with ISO 7198 8.2.3 “Determination of integral water permeability/leakage.”

The both ends of the sample graft and the adaptor were sealed to prevent water leakage and fixed. The internal lumen of the graft was filled with reverse osmosis (RO) water to add the internal pressure of 120 mmHg, and the volume of water leakage was measured for a given period. The pressure was measured by a pressure gauge (PG-200-102G-P, Nidec Copal Electronics Corporation, Tokyo, Japan), and the time was measured by a timekeeper (S321, Seiko Watch Corporation, Tokyo, Japan). For obtaining the volume of water leakage per unit of time, measurements were divided by the surface area of the used sample graft. The volume of water leakage was obtained by the measurement with an even balance (FX-3200, A&D Company Limited, Tokyo, Japan) and the subsequent conversion based on the equation, 1 gram = 1 mL. The value was divided by the surface area of the sample graft, and the result (mL/cm²/min) was recorded.

**Water leakage from the needle puncture**

The sample graft was cut open to make a test piece of 2-cm square sheet, which was mounted on the holder designated by ISO 7198: 1998 8.2.2 “Determination of water permeability.” The pressure of 120 mmHg was added to the test piece, and the volume of water leakage per minute (b) was measured [the weight of water was measured and the measurement (gram) was converted into milliliter (1 gram = 1 mL)]. Subsequently, the test piece was removed, and a vascular clamp (20 cm Cooley Tang. Occl. Clip, BOSS) was applied at 7/9 notch for 120 minutes. After the clamp had been released, the volume of water leakage (a) was measured in the same procedure as the measurement of (b), and the volume of water leakage following use of a clamp was calculated by subtracting (b) from (a). For the control grafts, the measurements were made in the same procedure. For the measurement of pressure, time, and weight, we used a pressure gauge (PG-200-102G-P, Nidec Copal Electronics Corporation, Japan), timekeeper (S321, Seiko, Japan), and even balance (FX-3200, A&D, Japan), respectively.

**Water leakage following use of a clamp**

The sample graft was cut open to make a test piece of 2-cm square sheet, which was mounted on the holder designated by ISO 7198: 1998 8.2.2 “Determination of water permeability.” The pressure of 120 mmHg was added to the test piece, and the volume of water leakage per minute (b) was measured [the weight of water was measured and the measurement (gram) was converted into milliliter (1 gram = 1 mL)]. Subsequently, the test piece was removed, and a vascular clamp (20 cm Cooley Tang. Occl. Clip, BOSS) was applied at 7/9 notch for 120 minutes. After the clamp had been released, the volume of water leakage (a) was measured in the same procedure as the measurement of (b), and the volume of water leakage following use of a clamp was calculated by subtracting (b) from (a). For the control grafts, the measurements were made in the same procedure. For the measurement of pressure, time, and weight, we used a pressure gauge (PG-200-102G-P, Nidec Copal Electronics Corporation, Japan), timekeeper (S321, Seiko, Japan), and even balance (FX-3200, A&D, Japan), respectively.

**Change in luminal diameter following pacing stress**

Each of two Triplex® grafts was cut into 6 pieces. After pieces of the both grafts had been fully mixed, they were divided into two groups of 6 samples. One of the groups was used as the baseline group, each of the sample pieces was cut open in a longitudinal direction, and the circumferential length was measured using a ruler. In accordance with the FDA guidance 14) 12.7, “Data acquisition analysis for durability testing,” the other group of samples was soaked in 37°C physiological saline, and 100 million cycles (1 sample), 200 million cycles (1 sample), 300 million cycles (1 sample), and 400 million cycles (3 samples) of pacing stress were applied at 80/160 mmHg. Subsequent to the application of stress, the circumferential length of each sample was measured. The pacing cycle was 25 Hz.
Water leakage following use of a clamp

The result is shown in Table 3. For control grafts, about 1–3 mL/min increase in water leakage was observed per unit area (cm²) following use of a clamp. For Triplex grafts, no increase in water leakage was observed following use of a clamp.

Change in luminal diameter following pacing stress

The result is shown in Fig. 2. The result of the analyses following these tests indicated that there was a significant difference between baseline and the figures after 100 million cycles of pacing stress, but no diameter enlargement was observed over time or following the pacing stress of 400 million cycles.

Discussion

The properties required for large diameter vascular grafts used in the thoracic region or abdominal region include low blood permeability from the vascular graft and operability that allows easy suture during the operation as well as postoperative histocompatibility and long-term patency.\(^{15,16,17,18}\)

Currently, typical vascular grafts intended for use in aorta region are those coated with bovine-derived collagen.
middle layer made of elastomer, which may be beneficial in terms of the life of the grafts.

In addition, Triplex® grafts experimentally demonstrated a good sealing ability following the penetration of a suture. Based on the result, we expect that Triplex® grafts may contribute to the reduction of bleeding following the anastomotic operation in clinical settings.

In practice, it is hard to expect that the results of this study would be reflected completely in a clinical use, based on the types of the surgical equipment and the operation procedures. However, we believe that further evaluations under different conditions will reveal more of the advantages and disadvantages of the Triplex® grafts.

At burst strength testing, higher measurements were found in the control grafts regarding the grafts having the internal diameter of 26 mm. However, the result of a separate in vitro accelerated aging test indicates that the measurements go into reverse over time within the product life of the grafts (half strength). Based on this, we consider that the long-term change of strength measurements must have more significant meaning than the absolute values shown by the results of this test.

The measurements of circumferential tensile strength were greater in the control grafts regarding the grafts having the internal diameter of 26 mm. The reason of this result may include the difference in the structure (knitted graft and woven graft). However, because the minimum tensile strength required is considered to be approx. 0.25 N/mm when taking into account the actual circumferential tensile stress caused by the blood pressure,2) we believe that the longitudinal tensile strength of the Triplex grafts is at a sufficient level for both 8-mm (approx. 11 N/mm) and 26-mm (approx. 18 N/mm) diameters.

In comparisons of the suture retention strength, for the grafts having the internal diameter of 26 mm, all the results were greater in the control grafts except those of oblique-90° test. The load applied on the anastomotic site was calculated according to the equation described in the “Suture hole elongation” section above, and the value per needle at the blood pressure of 200 mmHg is 0.018N for 8 mm diameter grafts and 0.017N for 26 mm diameter grafts. All the measurements in this study are considered to be 18N or greater.

Testing on the change in luminal diameter following pacing stress was conducted this time in accordance with the FDA guideline, which states that 400 million cycles are equivalent to approx. 10 years in clinical settings.
The result of the analyses following these tests indicated that there was a significant difference between baseline and figures after 100 million cycles of pacing stress, but no diameter enlargement was observed over time or following the pacing stress of 400 million cycles.

The pacing stress was followed immediately by a temporal dilation of diameter that might be associated with the application of internal pressure. Therefore, no diameter enlargement was seen over time and following 400 million cycles of pacing stress. In actual clinical practice, complex associations with a vascular graft must exist including those of biological stress, chemical stress, and multiple factors as well as physical stress. Thus, further studies are needed for this test item.

**CONCLUSION**

In order to demonstrate the fundamental properties of novel Triplex® grafts, we conducted physical property tests with reference to the international guidelines for vascular graft.

1. The results of the tests experimentally demonstrated that Triplex® grafts led to less leakage from the needle puncture and/or the body of the graft compared to collagen coated vascular grafts.
2. The results of the tests showed that Triplex® grafts had strength comparable to that of the conventional vascular grafts.
3. With these results, it was experimentally shown that newly developed Triplex® vascular grafts have fundamental physical properties comparable to those of the conventional vascular grafts that are used widely in clinical settings.
4. Triplex® is expected to play its role as a clinically beneficial next-generation vascular graft.

**DISCLOSURE STATEMENT**

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None of the authors have any financial or other potential conflicts of interest to declare.

**REFERENCES**

14) FDA guidance for the preparation of research and applications for vascular graft prostheses, 1993.
18) Morota T, Yamaguchi A, Murata S, et al. Results of