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Algin-Impregnated Vascular Graft
Ⅱ. Preliminary Animal Study

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=Abstract=

Microvel ®double velour graft impregnated with a biodegradable algin was studied as a new vascular graft. It is impervious to blood but still retains high porosity. This graft does not require preclotting during implantation and has good tissue ingrowth and biological healing properties. Two vascular grafts impregnated with algin (6mm in diameter) were implanted in the aorta of mongrel dogs without preclotting. Two identical grafts were preclotted and served as controls. The grafts were harvested 2 and 4 months postoperatively, and the healing pattern was examined by a light microscope after hematoxylineosin staining. It was observed that endothelial cells were incompletely covered on both algin-impregnated and control grafts after 2 month implantation, while they were fully covered on both grafts after 4 month. There were no significant differences in subendothelial granulation tissue organization and fibrinoid material absorption between the algin-impregnated and control grafts. The algin-impregnated graft did not show any harmful effect on the healing and thus can be a new promising graft which is not necessary preclotting during the implantation.

1. INTRODUCTION

It has been commonly recognized that the porosity of vascular grafts plays an important role for their long-term patency and overall biological performance[1]-[8]. The porosity of the vascular grafts allows easy handling and anastomosis, and good flexibility. It also facilitates transmural ingrowth of connective tissue into the grafts and better healing into the surrounding tissue. Therefore, the porosity is an essential component for long term function of vascular grafts. The main disadvantage of highly porous vascular grafts is their high permeability for blood during implantation. It may result in severe blood leakage through the graft wall. Thus the grafts must be preclotted with blood before implantation to obtain zero permeability. Geneally the grafts are immersed in or flushed with fresh blood of patient. But this preclotting process is often time-consuming, cause blood transfusion, and may lead to increased usage of the patient's blood. In the case of an emergent patient with large bleed-

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ing by an accident, it may be fatal. It is also dangerous when the patient has been systematically heparinized for surgery.

Many research works have been done to develop new vascular grafts, which are blood tight during implantation and thus eliminate the need for preclotting the grafts, and become sufficiently porous to facilitate tissue ingrowth and biological healing. Most commonly used methods include coating or impregnation of the porous graft with a biodegradable component. The coated or impregnated vascular graft protects blood leakage during the implantation. Due to its gradual degradation and dissolution in the body, the resorbable material creates increasingly large pores in the initially impervious graft, allowing the ingrowth of periprosthetic tissue[9]. Until now, various proteins have been used as the biodegradable components for coating or impregnation of the grafts. They include albumin [10]-[21], gelatin or elastin[22]-[29], collagen [6][30][43], and fibrin[38][44]. The vascular grafts pre-treated with these proteins showed little blood leakage and faster healing compared to the grafts preclotted with blood. However, the proteins are generally unstable, hard to handle, very expensive, and not easy to make compatible with usual storage and sterilization procedures.

The objective of this study was to develop and evaluate a new vascular graft which is impervious to blood but still retains high porosity for tissue ingrowth and biological healing. A porous, knitted polyester(Dacron®) graft was selected as a control graft. It was impregnated with a non-proteinaceous material, algin, which is biodegradable and biocompatible. The algin was filled into the interstices of the graft fabrics and cross-linked each other to be stably deposited onto the graft.

In the previous work[45], the algin-impregnated vascular graft was characterized in vitro. The surface chemical structure and morphology, water permeability, coating weight, mechanical properties, and whole blood clotting time of the algin-impregnated graft were determined and compared to those of the control one. In this study, the algin-impregnated vascular graft was implanted in the aorta of mongrel dogs without preclotting and the healing pattern of the graft was compared to that of the preclotted control one.

2. MATERIALS AND METHODS

Description of Selected Vascular Graft

A porous, knitted polyester (Dacron®) graft, i. e. Microvel® double velour graft (Meadox Medicals, Inc., Oakland, NJ, USA) with 6 mm in diameter, was selected for this study. It has a nominal wall thickness of 0.58mm, approximate interior velour height of 0.20mm, and exterior velour height of 0.37mm.[46][47]

2 · 1 Algin Impregnation Treatment of the Graft

Algin, which is also called sodium alginate or alginic acid sodium salt, was impregnated to the Microvel double velour graft. For this, the graft surface was oxidized in sulfuric/chromic acid solution. Then, the graft was immersed in 1 w/v % algin solution and degassed in vacuum oven. This step provided impregnation of algin into the interstices of the graft. After that, the graft was taken out from the algin solution and immersed in 2 w/v % calcium chloride ($CaCl_2$) solution. By this treatment, the impregnated algin molecules were cross-linked each other. After washing with ethanol/water mixtures (80/20 v/v %), the graft was dried in vacuum oven. The detailed procedures for algin impregnation and characterization of the graft were reported in the previous paper[45].

2 · 2 Surgical Procedure

Four mongrel dogs, weighing between 10 and 15 kg, were prepared for vascular surgery. After shaving upper extremity and abdominal areal followed

by povidone-iodine soap scrub and draping, sodium pentothal, 25 mg/kg, was intravenously infused slowly into the dogs. Anesthesia was maintained with mixed oxygenated nitrous oxide gas and halothane.

The lower abdomen was incised and the internal organs were wrapped aside for more wide view of the aorta. After the full dissection of abdominal aorta and comon iliac arteries including the ligation of small branches, around the proximal and distal aortic tissues were wrapped with umbilical tape and nelaton rubber to ensure the closing of the lumen. After both proximal and distal sides of the aorta were clamped with straight and right-angled clamps, the cutting was done and the luminal size of the vascular graft was compared for anastomosis.

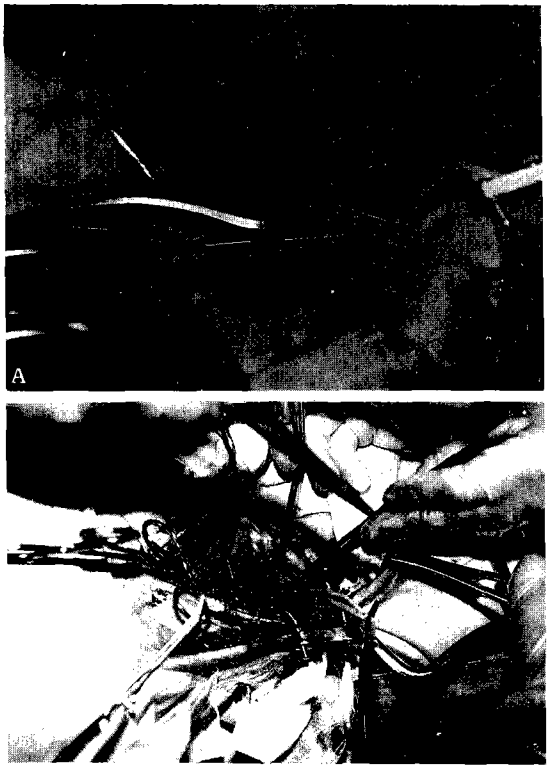

Fig. 1 Photographs showing (A) preclotting and (B) implantation processes of the control graft.

The dogs were heparinized (5,000 to 10,000 units) before implantation to prevent intravascular thrombosis. The control vascular grafts were implanted after preclotting with about 5 cc of fresh blood drawn from the proximal side of the aorta (Fig. 1), while the algin-impregnated grafts were implanted without preclotting. The grafts with 2 cm in length were anastomosed on the divided aorta in an end-to-end type with a 5.0 Prolene suture. The incision was closed layer by layer after hematosis was secured.

2 · 3 Postoperative Care

An antibiotic (Cefamezin, 1.0 gram) was injected intramuscularly before and after operation and continued daily until postoperative 5 days. During postoperative 5 days the wound was checked daily and femoral pulse was checked.

2 · 4 Pathologic Examination

The dogs were sacrificed 2 and 4 months postoperatively and the gross morphology of the anastomotic sites was examined. After the grafts were harvested to be contained both anastomotic ends, they were fixed in formaldehyde and embedded into paraffin block. The graft-embedded paraffin block was cut into 4-5 μm thickness using a microtome. After mounting on glass slides, they were stained with hematoxylin-eosin and glued to fix cover-glasses on the stained samples. The healing pattern of the stained samples was examined by a light microscope.

3. RESULTS

3 · 1 Algin Impregnation and Characterization of the Graft

The algin-impregnated vascular graft presented good conformability, although it showed mild rigidity and a little expanded crimping compared to the control one. The morphology examination of the

algin-impregnated graft confirmed that the algin was relatively uniformly deposited on the fabric surfaces, although some flaws existed. The weight of algin coating was 39.4 mg/g of the graft. The water permeability of the control graft was 1846±26ml/min · cm^2 at 120 mmHg and reduced to 14.7ml/min · cm^2 after algin impregnation. The mechanical properties of the graft were not changed by the impregnation treatment[45].

3 · 2 Pathologic Findings

Host tissue reactions of the grafts were evaluated at 2 and 4 months after implantation. The control graft after 2 month of implantation showed imcomplete lining of endothelial cells which extended only slightly from both ends (Fig. 2 (A)), while that after 4 month showed complete lining of endothelial cells (Fig. 2 (B)). Locally formed fibrin layer was appeared in the luminal side of the graft after 2 month, but in the later stage (after 4 month) the fibrin layer was almost absorbed. In subendothelial layer, infiltrated in flammatory cells were completely replaced with fibrous connective tissues after 4 month and well organized granulation tissues were formed. Thrombus formed in the early stage (2 month) was completely resorbed and well organized with granulation tissues and small capillaries after 4 month (Fig. 3). Inflammatory cells infiltrating around the outer layer of the grafte were mainly mononuclear cells (Fig. 4). The infiltration tendency decreased with time.

The algin-impregnated vascular grafts showed almost same histological reactions. Endothelial cells were lined in a same manner with the control grafts, i.g., incomplete lining after 2 month of implantation and complete lining after 4 month (Fig. 5). The thrombus formation on the algin-impregnated graft showed more wide and condensed pattern probably due to the algin action for clot formation. The formed thrombus was resorbed and replaced with granulation tissues, mainly fibroblast

Fig. 2 Endothelial cell lining on control vascular graft.
(A) After 2 month of implantation, endothelial cells were developed scantly on large thrombus body.
(B) After 4 month, thrombus was almost absorbed and endothelial cells were lined completely over fibrous connective tissues. (Original magnifications, ×100).

and elastic fibers, subendothelially. On the luminal side of the algin-impregnated graft, locally formed "Line of Zahn" implicates the continued cycling of the thrombus formation and resorbing process (Fig. 6). Neovascularization was actively formed in the well organized subendothelial layer (Fig. 6). Inflammatory response around the suture material and the graft was sustained still after 4 month but decreased with time.

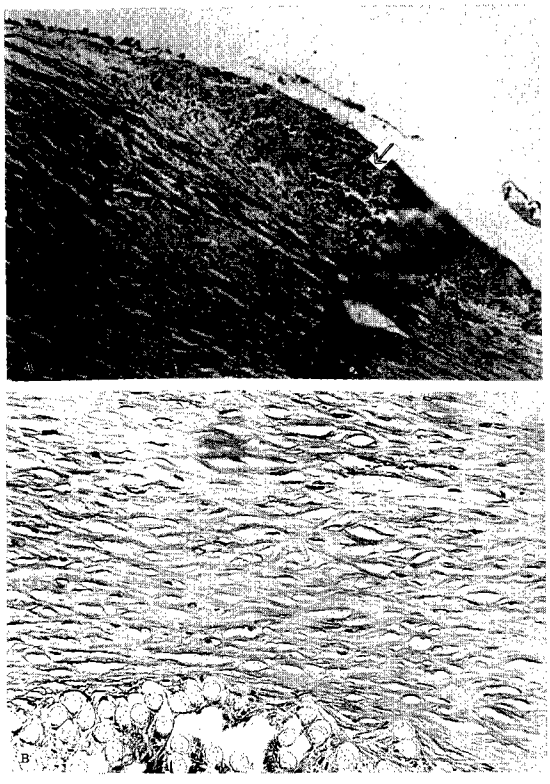

Fig. 3 (A) Thrombus formed (arrow) on control graft after 2 month of implantation. Thrombus was not completely absorbed on the luminal side of the graft and endothelial cells were lined over it.
(B) Well organized granulation tissues on control graft after 4 month. Granulation tissues were mainly composed of fibrous connective tissues and small capillaries.
(Original magnifications, ×200).

Fig. 4 Histological response of control graft after 2 month of implantation. There is diffuse mononuclear cell infiltration around vascular graft fibers. (Original magnification, ×200).

Fig. 5 Endothelial cell development on algin-impregnated vascular graft after 4 month of implantation. Homogeneous compact lining of endothelial cells was developed on well organized fibrous connective tissues. (Original magnification, ×100).

4. DISCUSSION

In this study, the histological response of the impervious polyester vascular graft impregnated with algin was compared to that of the porous one. The results of the preliminary animal study suggest that there are no significant differences concerning endo-

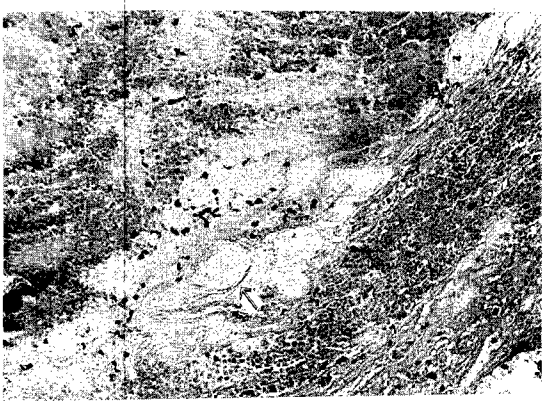

Fig. 6 Histological response of algin-impregnated vascular graft. Inflammatory cells were infiltrated inside of lumen thrombus. "Line of Zahn" (arrow) implicates the cycling pattern of thrombosis formation and resorption process. (Original magnification, ×200).

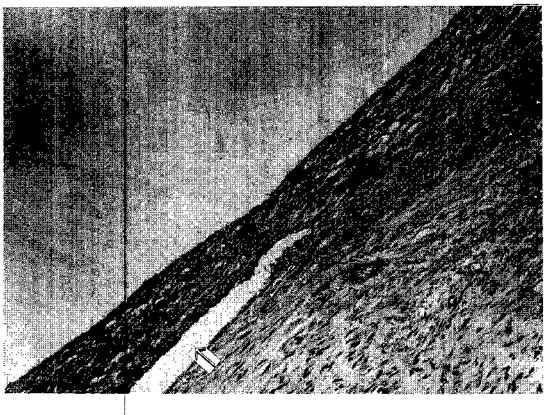

Fig. 7 Neovascularization (arrow) on algin-impregnated vascular graft after 4 month of implantation. Endothelial cell lining pattern and neovascularization were similar as that on control graft. (Original magnification, × 100).

thelial cell lining, granulation tissue ingrowth and neovscularization processes between the algin-impregnated and control grafts. The algin-impregnated graft offers important advantages over the control one, including the ability to be implanted without the need for preclotting. The algin-impregnated graft also offers advantages over protein-coated grafts, including easy handling and storage or sterilization procedures.

The preclotting process is often fastidious, time-consuming, and dangerous especially when the patient has been systematically heparinized for surgery. So the skipping of the preclotting process ensures more plausible operation. Also the infection is one of the greatest complications during vascular surgery, especially using foreign substances. Thus the use of the algin-impregnated graft is promising as a new vascular graft since it can reduce the chance of the contamination and skip the preclotting process.

The thickness and uniformity of the algin impregnated on the graft are thought to be very important parameters because the recurring thrombus formation may occur and cause severe blood flow obstruction and embolism to dismal parts. More controlled preparation for algin impregnation and extended animal study will be carried out to verify the performance of the algin-impregnated vascular graft.

5. CONCLUSIONS

In this study, we evaluated a new vascular graft which is impervious to blood but still retains high porosity. This graft did not required preclotting with blood during implantation.

A highly porous, knitted polyester (Dacron®) graft, Microvel® double velour graft with 6 mm in diameter, was impregnated with a biodegradable non-proteinaceous material, algin. The algin-impregnated graft were implanted in the aorta position of mongrel dogs without preclotting an the healing pattern of the graft was compared to that of the preclotted control one.

From the preliminary animal study, it was observed that endothelial cells were incompletely covered for both algin-impregnated and control grafts

after 2 month of implantation, while they were fully covered for both grafts after 4 month. There were no significant differences in subendothelial granulation tissue organization and fibrinoid material absorption between the algin-impregnated and control grafts. But the algin-impregnated graft after 4 month of implantation showed the continued cycling of thrombus formation and resorbing stages, which may be due to the algin action still remained on the graft.

The algin impregnation to porous vascular grafts can be a useful approach to prepare grafts which are blood tight during implantation and thus eliminate the need for preclotting process, but it seems that the amount of impregnated algin should be carefully controlled. The work to further control the algin impregnation and extend the animal study is on progress.

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