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Algin Impregnated Vascular Graft
I. In Vitro Investigation

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－**Abstract**－

Microvel double velour graft impregnated with a biodegradable algin was studied as a new vascular graft. It is blood tight but still retains high porosity. This graft does not need to be preclotted with blood before implantation and has good tissue ingrowth and biological healing properties. The algin impregnated vascular graft was investigated by "in vitro" tests in this study. It was characterized by ESCA analysis, SEM observation, and measurements of water permeability, algin coating weight, mechanical properties and whole blood clotting time. The water permeability of the graft was reduced more than 99% and the whole blood clotting time was fast more than three times by the algin impregnation treatment. "In vivo" performance examinations of the algin impregnated graft are on progress.

Introduction

It has been commonly recognized that the porosity of vascular grafts are very important.[1~7] The porosity of vascular grafts plays an important role for their long-term patency and overall biological performance. 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 high porosity 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. Generally the grafts are immersed in or flushed with fresh blood of patient to preclot the grafts. But the 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 bleeding 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 is blood tight during the imp-

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lantation. 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.[8] Until now, various proteins have been used as the biodegradable components for coating or impregnation of the grafts. They include albumin,[9~13] gelatin or elastin,[14~18] collagen,[5,19~25] and fibrin.[26] The vascular grafts pre-treated with these proteins showed little blood loss and faster healing compared to the grafts preclotted with blood. However, the proteins are generally not stable and thus they are not easy to make compatible with usual storage and sterilization procedures. They are also very expensive.

The objective of this study is to develop and evaluate a new vascular graft which is blood tight and has high porosity for tissue ingrowth and biological healing. We selected a porous, knitted polyester (Dacron) graft. It was impregnated with a non-proteinaceous material, algin, which is biodegradable and biocompatible. The impregnated algin was chemically bound on the graft fabrics and cross-linked each other to be stably deposited. The algin impregnated vascular graft was investigated by "in vitro" examinations in this study and its "in vivo" performance will be evaluated in the next study (part II).

Materials and Methods

Description of Selected Vascular Graft

A porous, knitted Dacron graft, Microvel® double velour graft (Meadox Medicals, Inc., Oakland, NJ, USA) of 6mm I. D. 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. The Microvel double velour graft was first commercially introduced in 1975. Since then, this synthetic vascular graft for arterial replacements has been used around the world.

The term "velour" describes a prosthesis with loop pile structures lining the exterior surface, interior surface, or both surfaces.[27] Vascular grafts made of double velour Dacron fabric display well defined fiber loops on both surfaces, the interior loops being the shorter. The Microvel double velour is a warp knit fabric. All warp knit graft fabrics are made with two or more strands of yarn that form an interlocking stitch which resists unraveling when the fabric is cut. The two-sided warp knit microloop pile of the Microvel double velour graft imparts a discernible softness and pliability. It readily accepts sutures, may be cut or beveled without fraying, and complies with ease to the host blood vessel.

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. First, the Microvel double velour graft was immersed in oxidation solution at 60℃ for 10 min to oxidize the fabric surfaces of the graft. The oxidation solution was prepared with conc. sulfuric acid (H_2SO_4), 30 wt%, chromic oxide (Cr_2O_3), 30 wt%, and purified water (using MPI ultra-pure water system), 40 wt%.

After washing with water, the oxidized vascular graft was immersed in 40% sodium hydroxide (NaOH) solution at 50℃ for 30 min to substitute carboxyl group produced on the fabric surfaces to the salt form. It was thoroughly washed with water until the pH value of the water solution after washing approaches 7 to 8.

Then, the graft was immersed in 1% sodium alginate solution and carefully degassed in vacuum oven. This step provides impregnation of algin to the graft. After that, the graft was removed from the sodium alginate solution and immersed in 2% calcium chloride ($CaCl_2$) solution for 5 min. By

this treatment, the impregnated algin molecules are cross-linked each other and some of them are chemically bound to the fabric surfaces of the graft. After washing with ethanol/water mixtures (80%/20%), the graft was dried in vacuum oven.

Characterization of Untreated and Algin Impregnated Grafts

ESCA analysis. The chemical structures of the Microvel double velour grafts, untreated, oxidized, and algin impregnated grafts, were investigated by electron spectroscopy for chemical applications (ESCA, ESCALAB MK II model, V. G. Scientific Co.), which is also called X-ray photoelectron spectroscopy (XPS). The radiation source was Mg Ka at 1253.6 eV and the power at anode was 300W. The interior and exterior surfaces were analyzed from the survey scan spectra.

Morphology. The interior and exterior surfaces of the grafts, before and after algin impregnation, were examined by scanning electron microscopy (SEM, JSM-840A, Jeol Co.). The specimens were gold deposited to improve their conduction and facilitate observation of fabric and yarn structures, surface morphology of the fabrics, and aspect of algin deposition on the graft surfaces.

Water permeability. The measurement of water permeability (often referred to as porosity) was carried out according to the technique described by Guidoin, et al.,[6] which gives an index of the interstitial leakage rate of the graft. The water permeability of the untreated and algin impregnated grafts were measured under a pressure of 120 mm Hg.

Coating weight. The coating weight of the algin impregnated graft was determined gravimetrically. The segment of the coated graft was dried and weighed. Then it was washed with water, stirred in 10% sodium acetate solution for 1 hr, and washed again with water several times. This process ensured complete removal of the algin (calcium alginate) deposited on the graft. After the graft was thoroughly dried, it was weighed again and the weight was compared with that of the algin impregnated one.

Mechanical properties. Tensile strength and elongation at break of the vascular grafts, before and after algin treatment, were measured using an Instron tester (ELE International Ltd.) at the crosshead displacement speed of 10 cm/min. Five axial and circumferential specimens were taken from the graft for the testing.

Whole blood clotting time (WBCT). WBCT is a global test of blood reactivity. The test was conducted with whole blood drawn without anticoagulant according to Lee and White method.[28] The clotting time on the untreated and algin impregnated graft surfaces were compared.

Results and Discussion

Algin Impregnation Treatment of the Graft

The reaction chemistry and conditions occurred on the Microvel double velour graft fabric (Dacron ; polyester) surface by algin impregnation treatment are schematically shown in Fig. 1. By the sulfuric/chromic acid solution treatment, carboxyl group is introduced on the fabric surface as evidenced by ESCA analysis.[29,30] The carboxyl

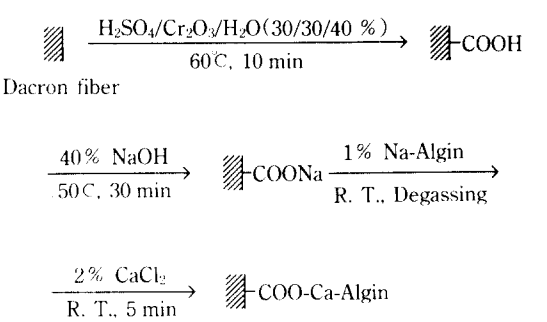


Fig. 1 Reaction scheme for algin impregnation.

group attached on the surface is substituted to sodium carboxylate group by the reaction with sodium hydroxide. Then the algin is adsorbed on the surface from the solution. The algin molecule also contains sodium carboxylate groups in its structure.[31] The algin molecules adsorbed are chemically grafted on the fabric surface or crosslinked themselves via calcium linkages in calcium chloride solution.

The algin impregnated vascular graft presented good conformability, although it showed slightly more rigidity and a little expanded crimping compared to its untreated one (Fig. 2). The mild rigidity of the graft can be further softened by glycerol treatment.[32]

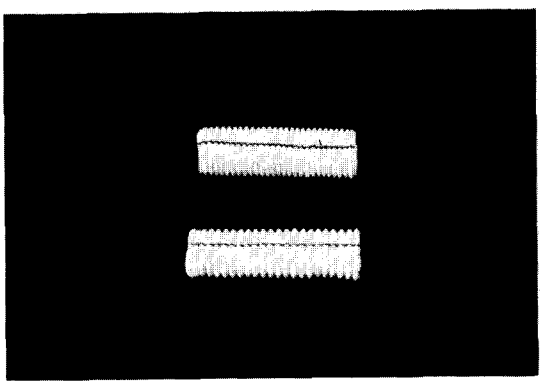


Fig. 2 Photograph of untreated (upper) and algin impregnated (lower) vascular grafts.

Characterization of Untreated and Algin Impregnated Grafts

ESCA analysis. ESCA is generally regarded as an important and key technique for the surface characterization and analysis of biomedical polymers. It provides a total elemental analysis (except for hydrogen and helium) and chemical bonding information of any solid surface which is vacuum stable. It is a nondestructive and surface sensitive (10 to 200 Å of the top) technique. Table 1 shows the results of ESCA analysis. The values for the surface composition of the vascular grafts, untreated, oxidized, and algin impregnated ones, were listed in the Table. The Microvel double velour graft is made by polyester fiber and showed only carbon and oxygen on its interior and exterior surfaces. As the graft was oxidized by the sulfuric/chromic acid solution treatment, it showed increased oxygen content. The exterior surface was slightly more oxidized than the interior. The algin impregnated surfaces showed more increased oxygen content than the oxidized ones due to the algin deposition (algin contains large oxygens in its structure). Both surfaces, interior and exterior, of the algin impregnated grafts contained calcium and sodium, which were derived from the reacted and the unreacted algins, respectively (refer to Fig. 1). The exterior surface showed higher calcium and oxygen contents and similar sodium content than the interior. This means that the algin was more highly reacted and deposited on the exterior surfaces than the interior.

Table 1. Surface composition (%) of untreated, oxidized, and algin impregnated vascular grafts

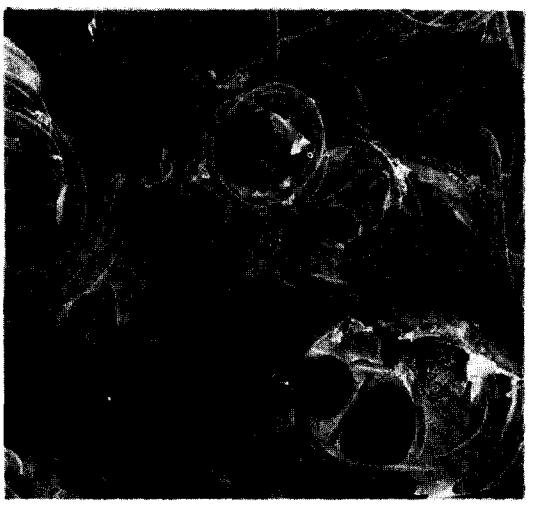
Surface	C	O	Ca	Na
Untreated				
Interior	69.9	30.1	--	--
Exterior	70.6	29.5	--	--
Oxidized				
Interior	62.7	37.3	--	--
Exterior	61.5	38.5	--	--
Algin impregnated				
Interior	54.2	42.9	1.3	1.6
Exterior	51.5	44.3	2.5	1.7

Morphology. Fig. 3 (A) and (B) show that the Microvel double velur graft had different interior and exterior fabric structures. Both surfaces displayed loop pile structures, the interior loops being

(A) Untreated, interior

(B) Untreated, exterior

(C) Algin impregnated, interior



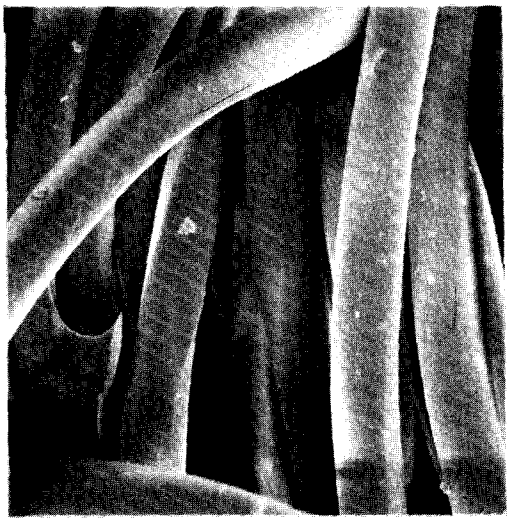
(D) Algin impregnated, exterior

Fig. 3 SEM pictures of the untreated and algin impregnated Microvel double velour grafts (×100, bar=100 μm).

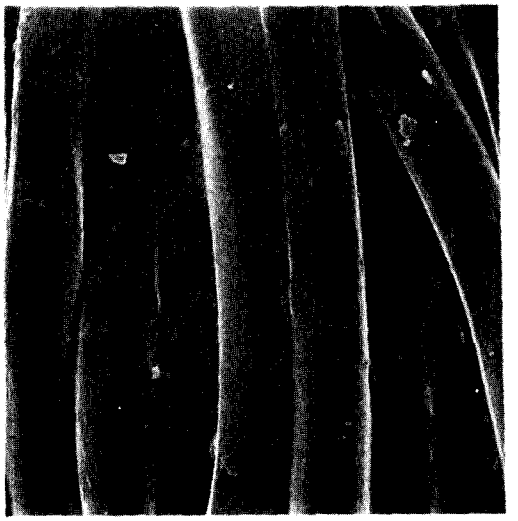
the shorter and well defined. Fig. 3 (C) and (D) show the surfaces of the algin impregnated graft. The SEM examination of the algin impregnated graft confirmed that the algin was well deposited on and penetrated into the surfaces, despite some flaws existed. The algin was more largely deposited on the exterior surface, which is agreed with the result of ESCA analysis. Fig. 4 shows enlarged pictures for the selected sections of the surfaces described in Fig. 3. The observed fiber diameters in Fig. 4 (A) and (B) were 13.8 to 15.4 μm. As seen in Fig. 4 (C) and (D), the individual fibers were surrounded with the algin.

Other characteristics. Table 2 compares some

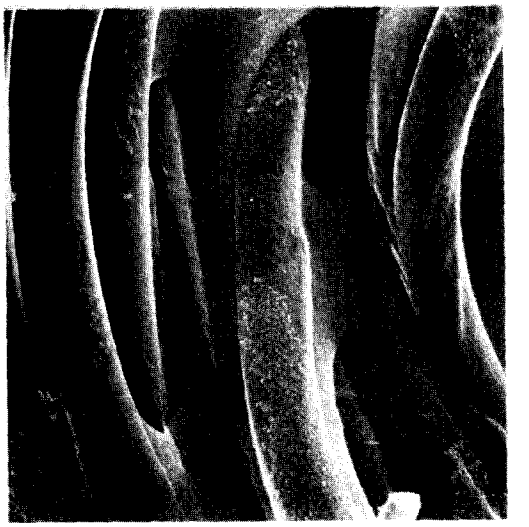
(A) Untreated, interior



(B) Untreated, exterior



(C) Algin impregnated, interior



(D) Algin impregnated, exterior

Fig. 4 Enlarged SEM pictures of the Microvel double velour grafts described in Fig. 3 (×700, bar = 10 μm).

characteristics of the untreated and algin treated Microvel double velour grafts. The water permeability of the graft was reduced more than 99% by the algin impregnation. The algin coating weight was 39.4 mg/g of the graft. The mechanical properties, tensile strength and elongation at break, of the algin impregnated graft showed similar trends to the untreated one. This means that the surface treatment carried out to oxidize the graft did not have any effect on the physical properties. The whole blood clotting time on the algin impregnated graft was much faster than the untreated one.

Table 2. Comparison of the properties of untreated and algin impregnated vascular grafts

Sample	Water permeability (ml/min · cm^2)[a]	Algin coating weight (mg of coating/ g of graft)
Untreated	1846 ± 26	— —
Algin impregnated	14.7 ± 2.4	39.4

[a]Measured at 120 mm Hg

Sample	Direction	Tensile strength (N/mm^2)	Elongation at break (%)
Untreated	Axial	12.4 ± 0.5	156
	Circumferential	13.0 ± 0.6	310
Algin impregnated	Axial	12.5 ± 0.5	170
	Circumferential	14.0 ± 0.7	310

Sample	Whole blood clotting time (min)
Untreated	8.1
Algin impregnated	2.6

Thus, we can expect that the blood are fast coated on the wall of the algin impregnated graft after implantation and the blood leakage is protected. Actually, we could observe that the algin impregnated graft implanted in the aorta position of a dog was totally blood tight, from the result of our preliminary "in vivo" examination.

Conclusions

From this study, we evaluated a new vascular graft which is blood tight but still retains high porosity. This graft does not need to be preclotted with bloood before implantation, which process is time-consuming and may cause blood transfusion. The high porosity of the graft provides good tissue ingrowth and biological healing.

We impregnated a highly porous, knitted polyester (Dacron) graft (i. e., Microvel double velour graft) with biodegradable non-proteinaceous material, algin. The algin impregnated vascular graft was evaluated by "in vitro" examinations. The algin was slightly largely deposited on the exterior surface of the graft than the interior as evidenced by the results of ECSA and SEM analysis. The water permeability of the graft was reduced more than 99% by the algin impregnation treatment. The oxidation treatment on the graft (which is required for the stable algin deposition) did not affect mechanical properties of the graft. We could observe that the graft surface impregnated with algin has much faster blood clotting time than the untreated one.

This vascular graft and control one with 6mm I. D. were implanted in dogs at the aorta position and "in vivo" experiments are performing now by the group of the Medical School at Seoul National University. The results will be discussed in the next study (part II).

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