Vascular grafts collagen coating resorption and healing process in humans

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ABSTRACT

Background: The objective of the present study was to evaluate the bioresorption rate of collagen coating (CC) sealed on textile vascular grafts (VGs) and their healing in humans using histologic analysis of explanted VGs.

Methods: A total of 27 polyester textile VGs had been removed during surgery from 2012 to 2020. The segments underwent histologic assessment. The CC bioresorption rate was assessed using morphometric analysis to determine the internal and external capsule thickness, inflammatory reaction degree, presence of neovessels, and endothelial cell layer.

Results: A total of 27 VGs were explanted from 25 patients because of infection (n = 5; 18.5%), thrombosis (n = 7; 25.9%), stenosis (n = 2; 7.4%), rupture (n = 4; 14.8%), aneurysmal degeneration (n = 3; 11.1%), revascularization (n = 4; 14.8%), or another cause (n = 2; 7.4%), with a median implantation duration of 291 days (interquartile range [IQR], 48-911 days). VGs with remaining CC (n = 7; 26%) had been explanted earlier than had those without (n = 20; 74%; 1 day [IQR, 1-45 days] vs 516 days [IQR, 79-2018 days]; P = .001). After 1 year, no remaining CC was detected on the analyzed VG sections. VGs implanted for <90 days had had a greater CC maximal thickness (63.90 μm [IQR, 0-83.25 μm] vs 0 μm [IQR, 0-0 μm]; P = .006) and a greater CC surface coverage (180° [IQR, 0°-360°] vs 0° [IQR, 0°-0°]; P = .002) than those implanted for >90 days. VGs implanted for >90 days had a greater external capsule thickness (889.2 μm [IQR, 39.6-1317 μm] vs 0 μm [IQR, 0-0 μm]; P = .002), a higher number of inflammatory mononuclear cells and giant cells (168 cells [IQR, 110-310 cells] vs 0 cells [IQR, 0-94 cells]; P < .0001) and a higher number of neovessels (4 [IQR, 0-5] vs 0 [IQR, 0-0]; P = .001) than those implanted for <90 days.

Conclusions: CC had a slow bioresorption rate in humans. Complete healing was never achieved, with no endothelial coverage observed. This finding implies that CC might not help graft healing. (JVS—Vascular Science 2022;3:193-204.)

Clinical Relevance: The objective of the present study was to evaluate the bioresorption rate of collagen coating (CC) sealed on explanted textile vascular grafts (VGs) and their healing in humans using histologic analyses. We found that CC had a slow bioresorption rate in humans. Complete healing was never achieved, and no endothelial coverage was observed. Therefore, we question their use in daily practice because the faster healing potential of CC VGs has been an argument advanced by manufacturers for their use. Knowledge of the mechanisms of graft healing is necessary to understand the success and failure of the current bypass grafts.

Keywords: Blood vessel prosthesis; Histology; Implants; Vascular grafting

Cardiovascular diseases are the leading cause of death in the world.[1] Their annual incidence will increase to 23.3 million worldwide by 2030,[2] and peripheral arterial occlusive disease is the most prevalent vascular disease worldwide.[3] Vascular surgery using open and endovascular procedures has evolved greatly to treat peripheral arterial occlusive disease in the past decades. At present,

the best conduits for vascular grafts (VGs) are autologous veins.[3] However, owing to their limited availability, the need for synthetic substitutes has been critical. Among the different materials used, expanded polytetrafluoroethylene and polyester have become widely accepted as synthetic substitutes owing to their high resistance to degradation in vivo.[4] Polyester VGs are indicated for

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the replacement of medium and large caliber arteries.[5,6] However, if used for smaller diameter arteries, polyester VGs will induce thrombosis and a compliance mismatch.[5,6] Their knitted structure with textured fibers has improved their long-term dimensional stability and encapsulation.[5] Moreover, their high porosity favors cell infiltration from the external surface, allowing for good graft anchorage in the surrounding tissues. However, polyester vascular grafts also have a high permeability to water and, thus, to blood, which can lead to significant blood loss during implantation.[7] To overcome this disadvantage, a preclotting step was performed with patient blood. Since the 1990s, textile polyester VGs have been sealed with bioresorbable coatings such as albumin, gelatin, and collagen. The latter are cross-linked with either formalin vapors, glutaraldehyde, and/or carbodiimide.[8] Albumin has been used as coating because of its easy production and low experimental thrombogenicity.[9] The natural presence of collagen in connective tissue, its chemotactic properties, and its degradation effects on fibroblasts account for its use as a VG coating.[10,11] Gelatin has been more widely used as vascular coating than collagen because of its easier production and lower cost and thrombogenicity.[12] Coated VGs provide some advantages compared with the use of a preclotting step because they are ready to use and limit blood loss. Thus, coated VGs reduce the need for blood transfusions and the risk of transmission of nosocomial diseases such as human immunodeficiency virus and hepatitis B virus.[13,14] Moreover, it has been suggested that coated VGs could ensure a better healing process in terms of tissue ingrowth and endothelial coverage through controlled speed of resorption of the coating. Graft healing corresponds to the reestablishment of a functional endothelial cell layer on the luminal surface and to sufficient graft integration in the adjacent tissues. Endothelial cell coverage helps to limit persistent bacterial colonization on the graft, and neovascularization plays a key role in the early healing process of arterial grafts.[15-19] A better healing process will improve graft biocompatibility and reduce the risks associated with thrombotic restenosis or induced by intimal hyperplasia.[20] In animals, the healing process has been similar to that of noncoated VGs, once the coating has been resorbed.[8,21-23] However, unlike many animal models, in humans, noncoated VGs will never completely heal for their full length.[7,24] Healing has most often been observed only at 1 or 2 cm from the anastomotic sites, with a luminal surface covered by a neointima composed of collagen and endothelial cells.[15,25] The remaining surface will be composed of fibrin without endothelial coverage. In addition to being incomplete, the healing process has also been shown to be delayed in humans.[7] A study of explanted albumin-coated VGs has highlighted that albumin bioresorption is delayed.[16] Albumin coating was still observed years after implantation in humans. In contrast, different in vivo studies have shown that all coatings were resorbed in dogs within <1 month, corresponding to better healing in animals.[21-23,26] The coating bioresorption rate and healing both depend on the nature and origin of the protein and the type of cross-linking agent.[13,27-29]

The aim of the present study was to analyze explanted VGs to evaluate collagen coating (CC) bioresorption and their healing process in humans. Explanted VGs were collected using the Groupe Européen de Recherche sur les Prothèses appliquées à la Chirurgie Vasculaire (GEPROVAS) graft-retrieval program. The GEPROVAS aims to better understand and characterize the complications associated with VGs, such as the tearing and rupture phenomena. The GEPROVAS vascular explant analysis platform collects explanted vascular implants from various partner hospitals and industries. In the present study, we focused on three knitted VG models coated with type 1 bovine collagen: Dialine II (Lemaitre Vascular, Inc, Burlington, MA), Polymaille Extra Thin (Perouse Medical, Ivry le Temple, France), and Intergard (Maquet, Inc, Wayne, NJ).

METHODS

Patient characteristics

Explanted textile polyester grafts coated with type 1 bovine collagen were analyzed as a part of the GEPROVAS collaborative retrieval program from 2012 to 2020. The clinical data related to each patient were collected, including age, gender, cardiovascular risk factors, indication for implantation, graft location, cause of graft removal, and implantation duration. The inclusion criteria were implanted textile polyester VGs coated with type 1 bovine collagen and a known implantation duration. Patients were excluded from the present study if the clinical information was insufficient.

Explanted VGs

A nonimplanted Polymaille Extra Thin VG (Perouse Medical) served as the collagen-coated control VG. A Gore-Tex standard-wall removable ringed VG (W.L. Gore

Table I. Sites, indications for implantation, and causes of explantation

Variable	No.
Implantation site (n = 27)	
Femoropopliteal	7
Femorofemoral	2
Cross femorofemoral	3
Iliofemoral	3
Aortobifemoral	7
Aortofemoral	1
Aortic bi-iliac	1
Axillobifemoral	1
Unknown	2
Implantation indication (n = 27)	
Aneurysm	4
Ischemia	19
Trauma	2
Unknown	2
Cause of explantation (n = 27)	
Infection	5
Thrombosis	7
Stenosis	2
Rupture	4
Aneurysmal degeneration	3
Revascularization (inflow or outflow requiring another surgery)	4
Other	2

& Associates, Inc, Newark, NJ), which had been explanted after 8 months of implantation, served as the non-CC control.

Graft harvesting, processing, and gross morphology

The collected specimens were graft sections and not the full-length VG. The explanted VGs were fixed and preserved in 4% formaldehyde solution. Once received at GEPROVAS, each explant was submitted to a standardized protocol for evaluation, starting with a gross examination and followed by digital image capture (Nikon D5100; Nikon France, Champigny-sur-Marne, France). Each inspection was recorded in reports containing observational commentaries and anonymized clinical data. The graft characteristics are presented in Table I.

Histologic preparation

Segments of the VG specimens removed at surgery were selected for histologic analysis and cut with a scalpel blade at one end. They were cut both longitudinally and horizontally. Next, they were sent to the Center of Biological Resources of Strasbourg University Hospital for embedding, microtome cutting (thickness of 4 mm), and staining (hematoxylin and eosin and Masson's trichrome).

Histologic assessment

CC assessment. The presence of CC was assessed histologically. The maximal thickness of the CC and the degree of surface coverage over the cross-sectional area (the entire circumference of the section corresponding to 360°) were measured using Zen Lite 2 software, blue edition (Carl Zeiss Microscopy GmbH, Jena, Germany) on histologic sections stained with Masson's trichrome stain (Supplementary Fig 1).

Graft healing. Graft healing was considered to have occurred in the presence of the reestablishment of a functional endothelial cell layer on the luminal surface and sufficient graft integration in the adjacent tissues.

VG encapsulation. The presence of internal and external capsules was assessed. The internal capsule corresponds to the tissue growing inside the luminal side of the graft, and the external capsule corresponds to the tissue growing on the outside. Both capsules will usually be composed of fibrous and adipose tissues. Internal capsule formation improves graft hemocompatibility and the external capsule enables graft encapsulation in the surrounding tissues. The capsule thickness was visually described as absent, thin, intermediate, or thick. The thickness was considered thin at 50 to 100 μm, intermediate at 100 to 500 μm, and thick at >500 μm. The thickness of the internal and external capsules was measured randomly 10 times and averaged. These measurements were performed using Zen Lite 2 software on transverse histologic sections stained with Masson's trichrome stain.

Chronic inflammation assessment. Chronic inflammation was evaluated as absent, low with few mononuclear cells, moderate with many mononuclear cells, or high with many mononuclear cells and giant cells. To quantitatively assess for chronic inflammation, the number of inflammatory cells was counted manually over 10 random areas of 0.25 mm^2 located next to the VG yarn on histologic sections stained with hematoxylin and eosin. These areas were defined using a grid pattern and Zen Lite 2 software (Supplementary Fig 2).

Neoangiogenesis assessment. The identification of the markers of the reconstruction phase, succeeding the cleaning phase of the healing process, was assessed. The cleaning phase corresponded to the granulomatous inflammatory phase of the inflammatory reaction. It consists of the formation of a granuloma composed of macrophages and lymphocytes, which ensures the cleansing of necrotic debris and pathogens by phagocytosis. As characterized by neoangiogenesis, the presence of neovessels was assessed.[30] The number of neovessels present in the internal or external capsule was counted manually over five random areas of 0.25 mm^2 on histologic sections stained with Masson's trichrome stain. These areas were defined with a grid pattern using Zen Lite 2 software. Bioresorption was analyzed using the CC maximal thickness and the degree of surface coverage
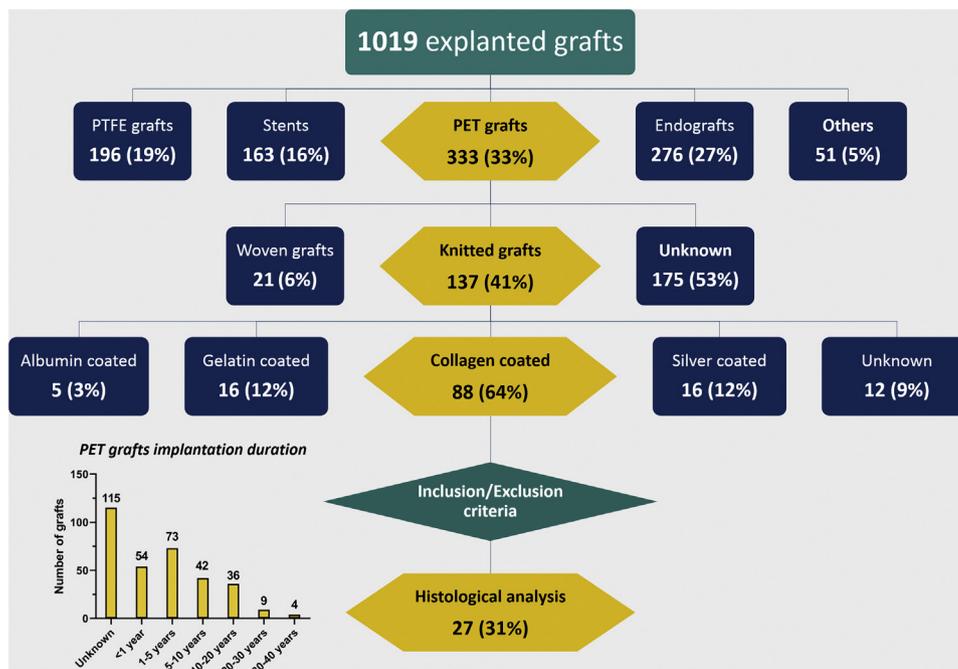
Fig 1. Flow chart of Groupe Européen de Recherche sur les Prothèses appliquées à la Chirurgie Vasculaire (GEPROVAS) graft retrieval program between 2012 and 2020 showing the number and type of explanted grafts collected through the graft retrieval program and the implantation durations of polyester explanted grafts collected between 2012 and 2020. *PET,* Polyester; *PTFE,* polytetrafluoroethylene.

determination. Healing was assessed by considering VG encapsulation, chronic inflammation, and neoangiogenesis. The CC presence, encapsulation, host immune reaction, and neoangiogenesis were compared between the short-term (<90 days; n = 11; 41%) and long-term (>90; n = 16; 59%) VG implantation duration and between infected and noninfected VGs.

Statistical analysis

Statistical analyses were performed using GraphPad Prism, version 8 (GraphPad Software, San Diego, CA), software. The quantitative variables were measured 5 times (ie, neovessel number) or 10 times (ie, maximal CC thickness, CC surface degree, inflammation cell number) and are expressed as median values. For two conditions with different data, the Mann-Whitney *U* test (a nonparametric test) was performed because the study data distribution was unknown. For more than two conditions with differing data, a Kruskal-Wallis test was used with Dunn's multiple comparison. A *P* value of < .05 was considered statistically significant.

RESULTS

Patient characteristics

A total of 27 VGs had been explanted from 25 patients (Fig 1). Of the 25 patients, 19 were men (76%) and 6 were women (24%). The mean age at implantation was 68 ± 13 years. In addition, 17 (74%) had had hypertension, 14

(67%) a history of smoking, 8 (36%) diabetes, and 12 (55%) dyslipidemia, and 6 (29%) were obese. The implantation sites, indications, and causes for explantation are reported in Table I.

Explanted VGs

A total of 27 explanted VGs were analyzed. Among them, 26 had been harvested at seven different university hospitals (ie, Strasbourg, Nantes, Saint-Quentin, Lyon, Blois, Grenoble, Lomme) and two private hospitals (ie, Aubagne, Colmar). One VG had been collected in the United States. Of the 27 VGs, 14 (52%) were Dialine II (Lemaitre Vascular), 8 (30%) were Polymaille Extra Thin (Perouse Medical), and 5 (18%) were Intergard (Maquet). Polymaille Extra Thin grafts are the only grafts to be free of formaldehyde and glutaraldehyde (Table I). The Intergard grafts were cross-linked with glutaraldehyde and Dialine II grafts with formalin vapor (Table II). The data set for the qualitative and quantitative analyses performed for each explant is summarized in Supplementary Table I. The duration of implantation ranged from 1 day to 14 years (median, 291 days; interquartile range [IQR], 48-911 days [ie, 9 months, 18 days]; Supplementary Table I). The median graft length was 44 ± 51 cm.

Graft analysis

CC bioresorption rate. Considering all VG brands, only 7 of 27 VGs (26%) still presented with CC. Of the 7 VGs

Table II. Collagen-coated vascular graft characteristics

Variable	Intergard	Dialine II	Polymaille Extra Thin
Coating	Type I bovine collagen	Type I bovine collagen	Type I bovine collagen
Cross-linking	Glutaraldehyde	Formalin vapors	NR
Construction	Knitted, external velour, reverse locknit	Knitted	Knitted
Water permeability <120 mm Hg, mL · cm^{-2} · min^{-1}	≤5	<10	≤5
Wall thickness, mm	0.49	0.53	0.32
Sterilization	NR	γ Irradiation	β Irradiation
Shelf life, years	NR	5	5

NR, Not reported.

with CC remaining, 6 (86%) had had an implantation duration of <90 days (Fig 2, *A*). The VGs implanted for <90 days showed a greater CC maximal thickness (median, 63.90 μm [IQR, 0-83.25 μm] vs 0 μm [IQR, 0-0 μm]; *P* = .006) and greater CC surface coverage (median, 180° [IQR, 0°-360°] vs 0° [IQR, 0°-0°]; *P* = .002) than those implanted for >90 days. Moreover, of the 11 VGs with an implantation duration of <90 days, 5 (45%) had not presented with any CC traces at all (Fig 2, *A*). VGs with CC traces had had a shorter implantation time than the VGs without CC (1 day [IQR, 1-45 days] vs 516 days [IQR, 79-2018 days]; *P* = .001; Fig 2, *B*). After 1 year of implantation in humans, no traces of CC were identified (Fig 3). The collagen had degraded quickly, one half of the VGs had had no CC at 90 days, and none had had CC at 1 year.

VG encapsulation. Of the 27 VGs, 16 (67%) had had an internal capsule and 16 (67%) an external capsule. Intermediate and thick internal capsules were identified even after a short implantation duration (<90 days), with thin and intermediate external capsules (Supplementary Figs 3, *A, and 4, A*). No differences were found between the implantation duration of the VGs with a thin, an intermediate, or a thick internal capsule (Supplementary Fig 3, *B*). The implantation duration of the VGs with a thick external capsule was longer than that for those without an external capsule (Supplementary Fig 4, *B*).

All seven VGs with an implantation duration of >3 years had had an external capsule. Six of the seven VGs also had an internal capsule (Fig 4, *A*; Supplementary Fig 3, *C*). VGs without capsules had been explanted earlier than had the encapsulated VGs (42 days [IQR, 1-253 days] vs 911 days [IQR, 256-2577 days]; *P* < .001; Fig 4, *B*; Supplementary Fig 3, *D*). VGs implanted for >90 days had a greater external capsule thickness (889.2 μm [IQR, 39.6-1317 μm] vs 0 μm [IQR, 0-0 μm]; *P* = .002). The external capsule thickness increased with increased implantation duration. In contrast, the internal capsule thickness did not vary with the implantation duration (Supplementary Figs 3, *A, and 4, A*).

Chronic inflammatory reaction. Local chronic inflammation was observed in 16 of the 20 VGs lacking CC (80%). Of the 27 VGs, 20 (74%) had white blood cells present in association with giant cells near the polyester yarn fibers (Fig 5, *A*). Moreover, the VGs explanted early had had lower degrees of inflammation and those explanted later had had high levels of inflammatory changes (Fig 5, *B*). VGs implanted for >90 days had had a higher number of inflammatory mononuclear cells and giant cells (168 cells [IQR, 110-310 cells] vs 0 cells [IQR, 0-94 cells]; *P* < .001). Chronic inflammation was seen with the VGs explanted later than those explanted earlier (467 days [IQR, 123-1452 days] vs 1 day [IQR, 1-18 days]; *P* = .005; Fig 5, *C*).

Neoangiogenesis. Of the 27 explanted VGs, the CC had been fully resorbed on 20 (74%), indicating the end of the cleaning phase, which corresponded to the granulomatous inflammatory phase of the inflammatory reaction. Of these 20 grafts, 5 (25%) had been explanted before 90 days and 15 (75%) after 90 days. Of the 27 VGs, 13 (48%) had neovessels present (Fig 6, *A*). VGs implanted for >90 days had a greater number of neovessels (4 [IQR, 0-5] vs 0 [IQR, 0-0]; *P* = .001) than those implanted for <90 days. The neovessels were essentially located in the external capsule with a variable distribution. The number of VGs without neovessels decreased with a longer implantation duration (Fig 6, *B*); thus, the VGs with a longer implantation duration had more neovessels (Fig 6, *C*).

Comparison between short- and long-term groups

Details of the comparisons between the short- and long-term groups are provided in the Supplementary Methods section.

DISCUSSION

In the present study, we performed a histologic analysis of 27 explanted textile polyester VGs that had been sealed with a CC. We found that only seven (26%) were still sealed with a CC applied to both external and
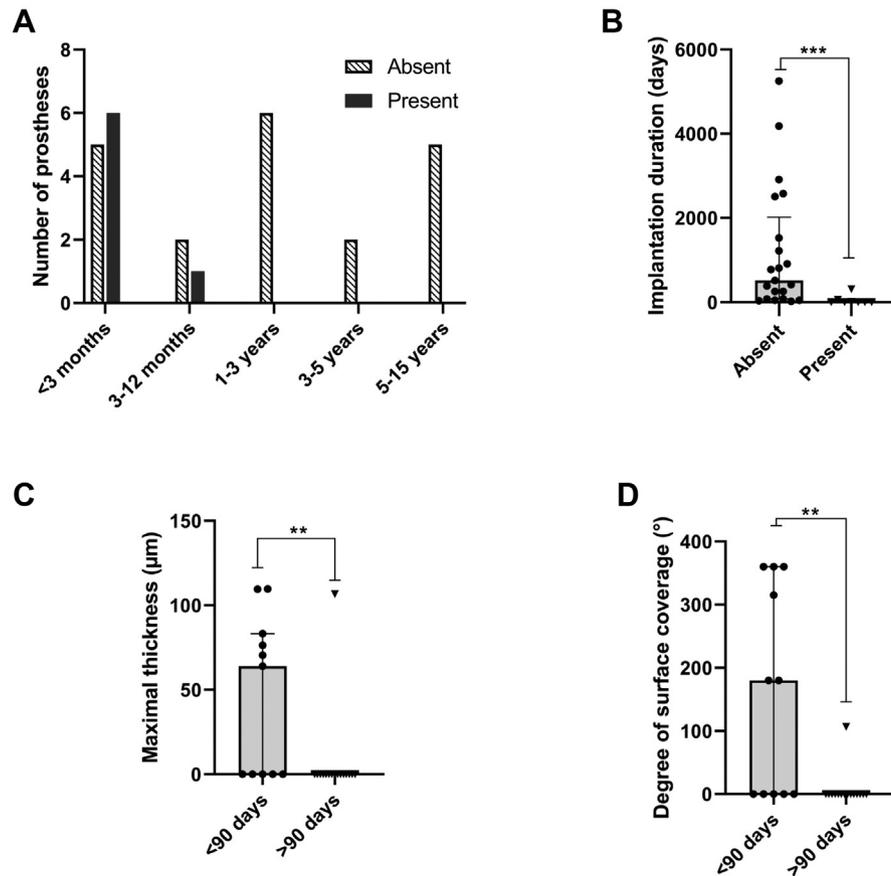
Fig 2. Collagen coating (CC) sealed on polyester vascular grafts (VGs) was resorbed within 12 months of implantation in humans with progressive degradation over time. **A,** Graph showing number of grafts with or without CC stratified by implantation duration. **B,** Graph showing implantation duration of grafts with (n = 7; 26%) and without (n = 20; 74%) CC. Morphometric analysis of CC with maximal thickness (**C**) and degree of surface coverage of CC (**D**) in the short-term (<90 days; n = 11; 41%) and long-term (>90 days; n = 16; 59%) implantation groups. Data presented as the median and interquartile range (IQR) and analyzed using the Mann-Whitney *U* test. ***P = .001 vs absent; **P = .001 vs >90 days.

internal surfaces at a median implantation duration of 1 day. The maximal thickness of the CC and its degree of surface coverage decreased with time. According to our explant-based analysis, the CC was still observed after 2 months, with a gradual degradation with time and remaining in trace amounts after 10 months of implantation in humans.

We noted a slow bioresorption rate of the CC, although the resorption was faster than that for the albumin coating because the albumin traces were still observed after 2 years in humans.[22] It is generally well recognized that the coating bioresorption rate will depend on both the nature and the origin of the protein and stability of the resulting cross-link.[31] Chakfé et al[7] suggested that the delayed bioresorption rate of albumin might have resulted from VG pretreatment with glutaraldehyde, which led to a very stable component that was slowly resorbed. In our study, only one of the three prosthetic models (Polymaille Extra Thin) was free of formaldehyde

and glutaraldehyde because the cytotoxic effect of these chemicals has been reported.[30] Unlike human albumin, the collagen used for the coating has a bovine origin; therefore, it might be recognized as a foreign body. Nonetheless, cross-linked collagen might still have low antigenicity owing to the formalin treatment, which has been reported for Dialine II.[31] Several studies have demonstrated the efficiency of glutaraldehyde and formaldehyde in reducing the antigenicity and cellular reactions of graft tissue.[14,32,33] The slow collagen bioresorption rate probably resulted from the low antigenicity of the cross-linked collagen and/or strong cross-linking properties. Moreover, nonspecific chronic inflammation was observed between the internal capsule and polyester yarn fibers of almost all the VGs. This inflammatory reaction might have influenced the collagen bioresorption rate.[34-37] However, the inflammatory reaction was assessed manually by one person, resulting in a relevant risk of bias, and the results could have been influenced
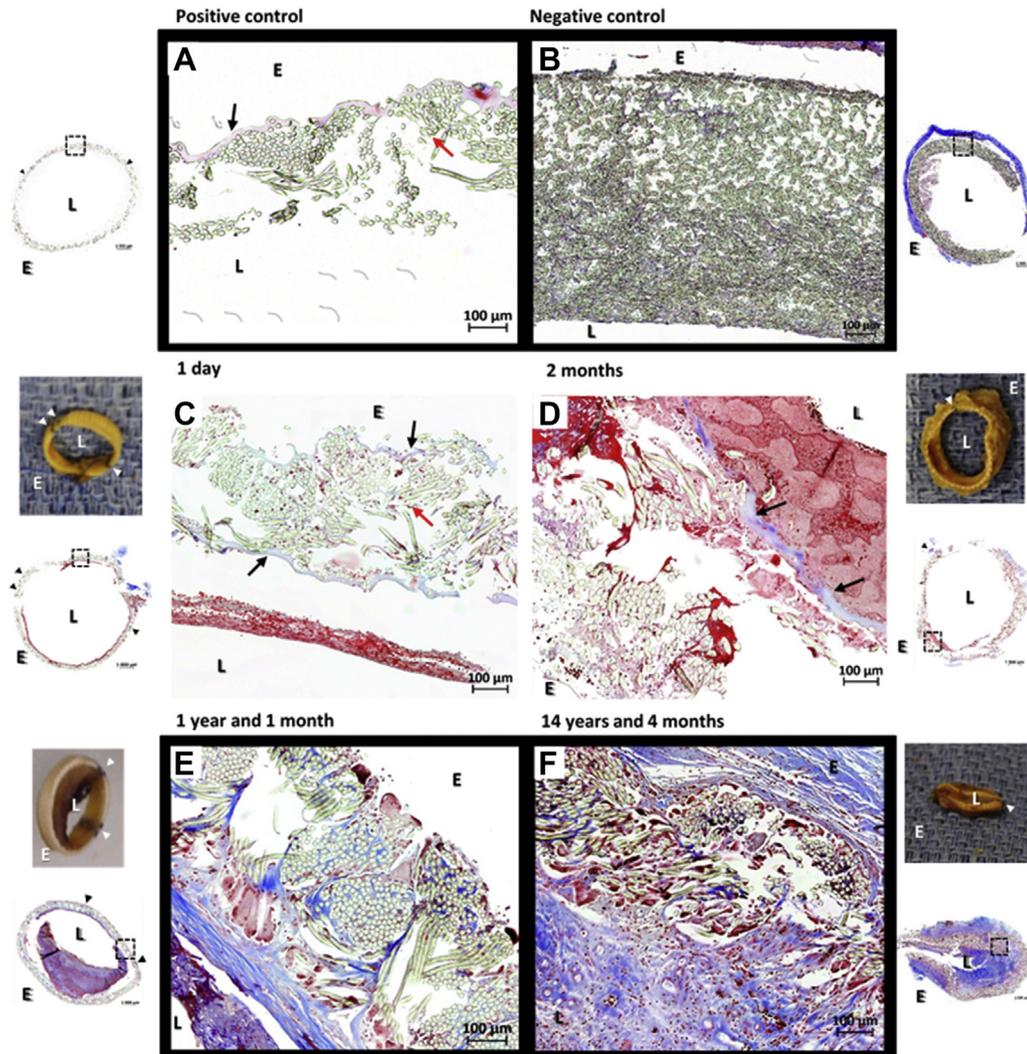
Fig 3. Representative photographs and photomicrographs of Masson's trichrome-stained transverse vascular explant sections (scale bar = 1000 μm). Specific regions of interest indicated by the *black dotted frames*, and *arrowheads* show guidelines on photomicrographs. Representative photomicrographs stained with Masson's trichrome of controls (*a*, *b*), Polymaille Extra Thin (*c*, *f*), Dialine II (*e*), and Intergard (*d*) explanted grafts after different implantation durations. *Black arrows* indicate collagen coating (CC). *Red arrows* indicate polyester yarn fibers. *E*, External side; *L*, luminal side. Objective ×10.

by external factors such as the timing of the infection and the use of antibiotics. It would be interesting to study the nature of the inflammatory reaction using protocols involving monoclonal antibodies against CD450, CD20, CD68 labeling T lymphocytes, B lymphocytes, and macrophages, respectively.

Compared with albumin-coated VGs, the healing sequence of VGs with CC was relatively poor.[16] No external encapsulation was present before 2 months after implantation, and no endothelial coverage was observed even after years of implantation. These findings have already been reported in humans.[38] However, the present study revealed encouraging findings. Tissue infiltration through the polyester yarn was observed for all explants with an external capsule present. Moreover,

neovessels were present in the perigraft connective tissue of 13 of the 16 VGs with an external capsule (81%). We also showed that the number of neovessels had increased with implantation duration. Therefore, adequate neovascularization seems to play an important role in the early healing process of arterial VGs.[15,17,18] However neoangiogenesis seems to occur relatively late in humans, although the CC seemed to be resorbed within 2 months. To further characterize neoangiogenesis and to confirm the absence of endothelial coverage on the graft luminal surface, specific immunostaining using antibodies against CD31 (protein of endothelial cell intercellular junctions) could be performed.

The healing characteristics are influenced by the presence of infection. Infection is known to induce a delay
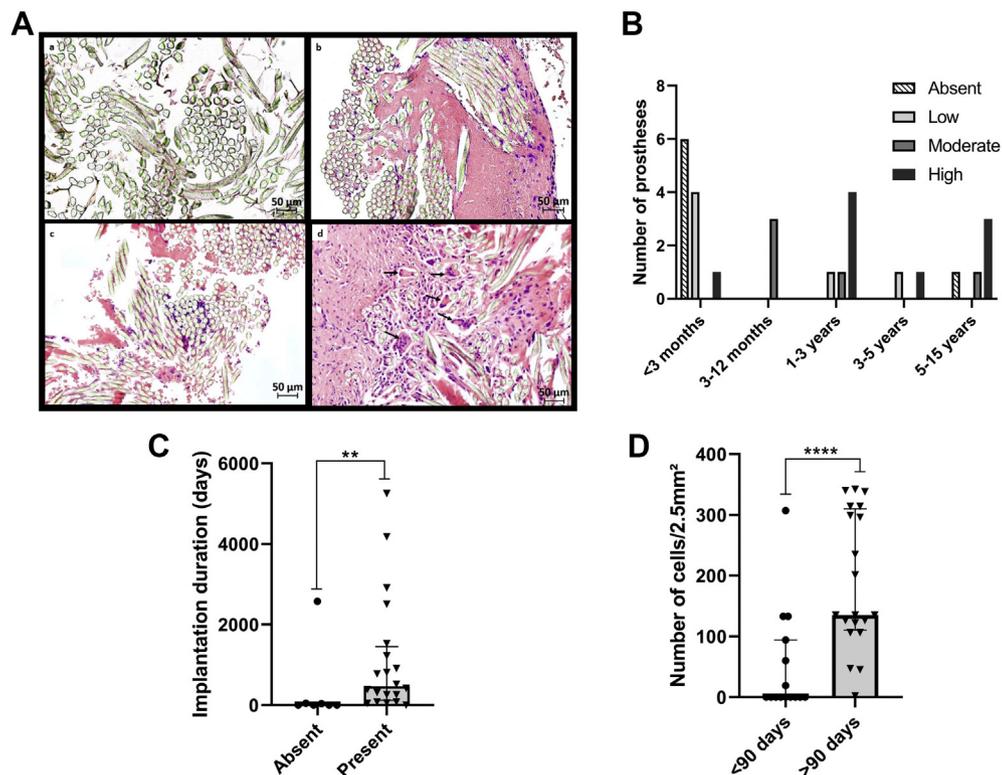
A

B

C
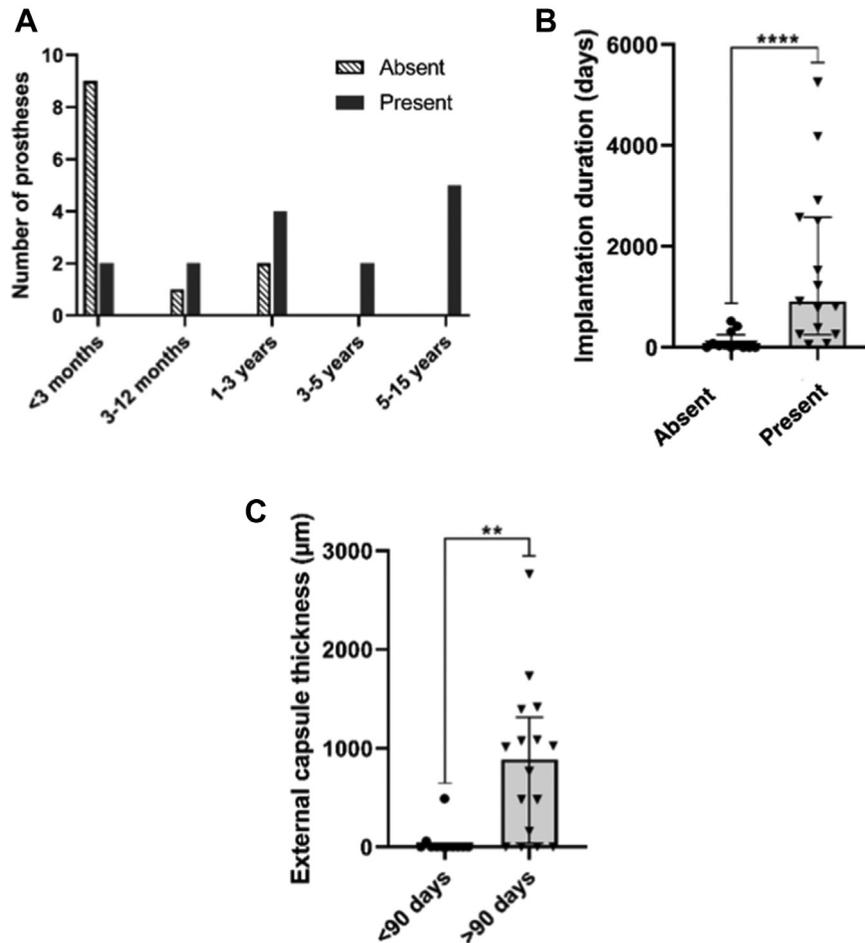
Fig 4. Polyester vascular grafts (VGs) without collagen coating (CC) remaining were more externally encapsulated in a collagenous tissue as the implantation duration increased. **A,** Graph showing number of grafts with or without an external capsule stratified by the implantation duration. **B,** Graph showing implantation duration of grafts with (n = 15; 56%) and without (n = 12; 44%) an external capsule. **C,** Graph showing results of quantitative analysis of external capsule thickness in short-term (<90 days; n = 11; 41%) and long-term (>90 days; n = 16; 59%) implantation duration groups. Data presented as the median and interquartile range (IQR) and analyzed using the Mann-Whitney *U* test. ****P < .001 vs absent; **P = .002 vs >90 days.

in synthetic VG healing in dogs.[18] One of the major consequences is the absence of incorporation by the surrounding tissue.[39] In our study, we found that the CC of the infected VGs was completely resorbed at shorter implantation durations than that for the noninfected VGs. Therefore, infection seems to influence CC bioresorption and might accelerate this process. Moreover, the presence of infection influenced external encapsulation because four of five infected VGs did not show any perigraft tissue. Chronic inflammation occurred earlier in the presence of infection, although the number of inflammatory cells was not greater than that for noninfected VGs. Only one of the five infected VGs (20%) had had neovessels.

Finally, our study, based on an explant-retrieval program, allowed us to study surgically removed specimens at different removal times. We found long-term effects and complications unforeseen by the results of

experimental evaluations, because the latter were rarely conducted for >6 months in animals.[21] The healing characteristics in dogs at 1 month are thought to correlate with healing in humans at 3 to 8 months.[27,40] Moreover, patients frequently have different comorbidities, such as tobacco use, hypertension, diabetes, or dyslipidemia, conditions difficult to reproduce in animal models.[17,41,42]

We found that the VGs with CC healed slowly, questioning their use in daily practice, because the faster healing potential of CC VGs has been an argument advanced by manufacturers for their use. Knowledge of the mechanisms of graft healing is necessary to understand the success and failure of the currently available bypass grafts. To the best of our knowledge, the present study is the second to analyze explanted coated VGs in humans and the first to investigate VGs with CC. Given the high number of vascular grafts performed per year, it is surprising how little attention has been given to healing
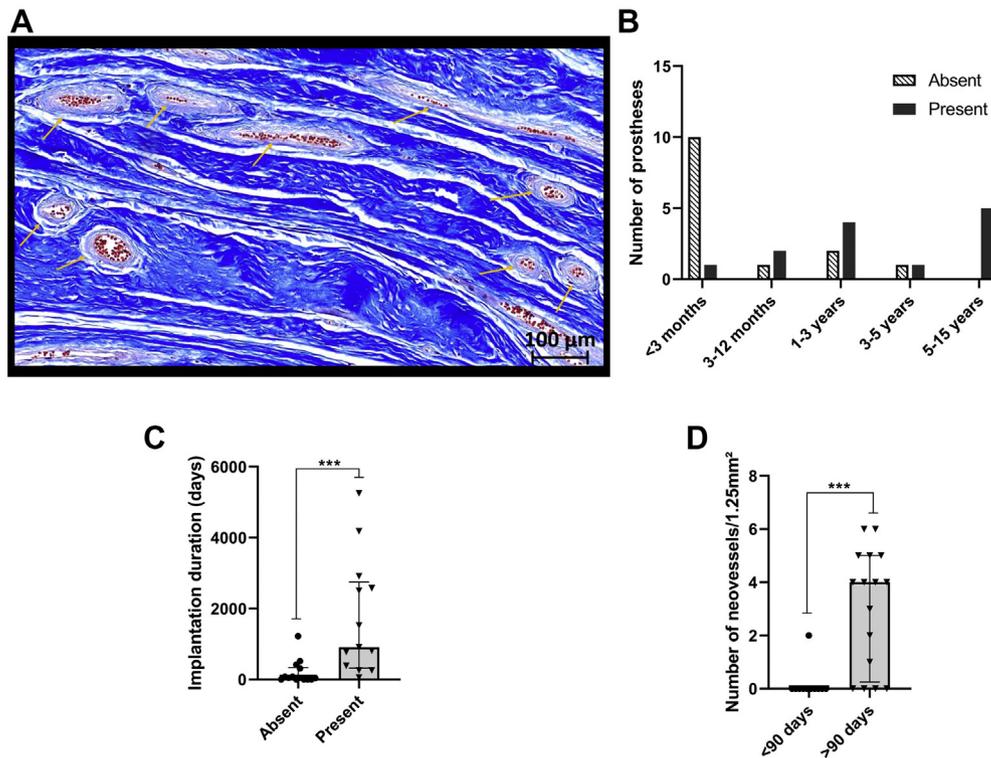
Fig 5. Polyester vascular grafts (VGs) without collagen coating (CC) remaining presented with greater chronic inflammation located between the polyester fibers as their implantation duration increased. **A,** Representative photomicrographs of hematoxylin and eosin-stained sections of one Intergard (*a*), two Dialine II (*b, c*) and one Polymaille Extra Thin (*d*) explanted grafts after 1 day (*a*), 1 month and 17 days (*b*), 10 months (*c*), 14 years and 4 months (*d*) of implantation (scale bar = 50 μm). These regions of interest illustrate different inflammation degrees: absent (*a*), low (*b*), moderate (*c*), and high (*d*) with giant cells (*black arrows*). **B,** Graph showing number of grafts with absent, low, moderate, or high inflammation stratified by the implantation duration. **C,** Graph showing implantation duration of grafts with (n = 20; 74%) and without (n = 7; 26%) chronic inflammation. **D,** Graph showing results of quantitative analysis of inflammatory cell number over a total surface of 2.5 mm^2 in the short-term (<90 days; n = 11; 41%) and long-term (>90 days; n = 16; 59%) implantation duration groups. Data presented as median and interquartile range (IQR) and analyzed using the Mann-Whitney *U* test. **P = .005 vs absent; ****P < .001 vs >90 days.

over the years. Although the initial enthusiasm for the availability of the polyester grafts led to several major studies in the 1960s and 1970s, relatively little has been added to our understanding since then. One main challenge of synthetic VGs is to reduce thrombosis and create an adequate blood flow surface. Because of their chemical composition or their roughness, synthetic grafts will be more thrombogenic compared to native arteries.[24,43] To reduce this thrombogenicity, a key solution would be to obtain a cellular contact surface with the blood on the grafts' luminal side. Ideally, polyester grafts should be sufficiently porous to allow for tissue ingrowth through the graft wall to provide a cellular and collagenous surface in contact with the blood.[38] Therefore, complete healing of VGs with an endothelial layer on the luminal surface would improve their hemocompatibility but also limit persistent bacterial colonization.[15] However, in our study, complete healing was not observed on the explanted sections without any endothelial layer

covering the luminal graft surface and potentially increasing the risk of thrombosis and infection.

Study limitations. A study using specimens from a retrieval program will have unavoidable limitations that must be considered when interpreting the results. Not all surgeons supplied their explanted VGs for investigation. In addition, only specimens, not the full-length VGs, were collected. Moreover, they might not exactly reflect the complications encountered daily. The second limitation of the present study was that the main source of material was surgically explanted specimens (ie, after thrombosis or infection). The healing characteristics of these VGs might have differed from patent VGs. Also, the surgical procedure for graft explantation could differ from one surgeon to another (and from one center to another) and might have altered the specimens. For example, the external capsule could have been detached during the surgical dissection. However, the

Fig 6. Neovessels, indicators of neoangiogenesis, were observed on polyester vascular grafts (VGs) without collagen coating (CC) remaining but with an external capsule. **A,** Representative photomicrograph of Masson's trichrome-stained sections of one Polymaille Extra Thin explanted VG after 14 years, 4 months of implantation showing neovessels in the external capsule (scale bar = 100 μm; objective ×10). Many blood cells were visible in the blood vessel lumen. **B,** Graph showing number of grafts with and without neovessels stratified by implantation duration. **C,** Graph showing implantation duration with (n = 13; 48%) and without (n = 14; 52%) neovessels. **D,** Graph showing results of quantitative analysis of neovessels number over total surface area of 1.25 mm^2 in the short-term (<90 days; n = 11; 41%) and long-term (>90 days; n = 16; 59%) implantation duration groups. Data presented as median and interquartile range (IQR) and analyzed using the Mann-Whitney *U* test. *E,* External side; *L,* luminal side. ***P = .001 vs absent; ***P = .001 vs >90 days.

dissections were all carefully performed. Moreover, six grafts were removed because of thrombosis; however, the internal thrombus was not analyzed, although the thrombus might have influenced the CC structure. This question remains open and might require future research. The number of inflammatory cells and neovessels were manually assessed by only one person. However, we believe that the repetition of counts should have been accurate enough to provide a correct number.

CONCLUSIONS

To the best of our knowledge, the present preliminary study is the first series of explanted collagen-coated VGs. The CC seemed to be resorbed within 2 months of implantation in humans, although traces after 10 months were observed in one case. Complete healing was never achieved, because no endothelial coverage was observed and the presence of internal encapsulation was quite variable. Our results have highlighted the need for more analyses of coated polyester VG explants to better understand their delayed healing in humans. Future

efforts are required to study a larger number of graft explants of the same model. It would also be interesting to develop immunofluorescence protocols to specifically detect polyester fibers and CC. Finally, future studies should investigate the inflammation and neoangiogenesis phenotypes in more detail.

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Final approval of the article: EH, SK, DD, FH, YG, FT, AL, NC
Statistical analysis: EH
Obtained funding: Not applicable
Overall responsibility: NC

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