

Comparison of the Antimicrobial Properties of Silver Impregnated Vascular Grafts with and without Triclosan

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WHAT THIS PAPER ADDS

This in vitro study compares two antimicrobial grafts containing silver or a combination of silver and triclosan, inoculated separately by four micro-organisms: *Staphylococcus epidermidis*, methicillin resistant *Staphylococcus aureus*, *Escherichia coli* producing extended spectrum beta-lactamase, or *Candida albicans*. The Synergy vascular graft combining silver with triclosan demonstrated better short-term antimicrobial activity compared with the silver graft for all micro-organisms tested. This study provides in vitro evidence that the new vascular graft combining silver and triclosan may be preferred to the silver only graft.

Objectives: The aim was to compare the antimicrobial efficacy of the silver impregnated collagen coated polyester vascular graft (IGS) with an identical graft combining silver and triclosan (IGSy).

Methods: This was an in vitro study. A non-antimicrobial collagen polyester vascular graft served as control (IG). The IG, IGS, and IGSy grafts were contaminated separately with inoculates of each of the following micro-organisms: *Staphylococcus epidermidis* (SE), methicillin resistant *Staphylococcus aureus* (MRSA), and *Escherichia coli* producing extended spectrum beta-lactamase (ESBL-EC) or *Candida albicans* (CA). MRSA, ESBL-EC, and CA were obtained from retrieved infected grafts. The in vitro antimicrobial efficacies of the contaminated grafts were evaluated by time to kill assays over a 24 hour period in accordance with CLSI Guideline M26-A. All assays were repeated six times. Bacterial survival numbers were obtained at 1, 4, 8, and 24 hours using a standard plate count procedure. Bactericidal activity was defined as a 3 log₁₀ reduction factor (logRF). To calculate the overall difference in the mean log₁₀ CFU/mL within 24 hours, a one way ANOVA with a Bonferroni correction was calculated separately for each graft.

Results: The IG graft showed an increase in the number of viable organisms for the four strains tested. IGSy offered better antimicrobial properties than IGS for both ESBL-EC and MRSA, since only the IGSy graft achieved > 3 logRF and fulfilled the standard criteria for bactericidal activity at 24 hours with 3.78 and 4.08 logRF, respectively. For samples inoculated with SE and CA, both antimicrobial grafts achieved 24 hour bactericidal activity with > 3 logRF. However, for CA the one-way ANOVA analysis demonstrated that the IGSy graft performed differently in terms of speed of antimicrobial action, appearing more active as early as 4 hours following inoculation ($p = .007$).

Conclusion: In the in vitro conditions, the Synergy vascular graft combining silver with triclosan demonstrated better short-term antimicrobial activity than the silver graft for all micro-organisms tested.

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INTRODUCTION

Aortic graft infections occur in less than 2% of prosthetic aortic reconstructions¹ but represent a severe complication with high morbidity and mortality rates. These frail infected patients,² sometimes admitted in hemorrhagic or septic shock, are exposed to an extensive debridement of all infected tissue, including (part of) the graft followed by an

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in situ reconstruction with a biological or prosthetic conduit.³ In order to restore lower limb perfusion, the use of non-prosthetic material such as deep femoral veins⁴ or cryopreserved arterial homografts⁵ seems a logical option if the aim is to prevent recurrent infection.⁶ However, cryopreserved arterial homografts are available in limited quantities⁷ and veins are not always suitable; moreover these techniques are not free from complications, for example the potential risks of delayed rupture,^{8,9} compartment syndrome and lower limb edema.^{10,11} For these reasons, prosthetic grafts still have a place. However, all antimicrobial solutions must be employed including secondary targeted antibiotic treatment as well as the use of antimicrobial grafts.³ Historically, Rifampicin soaking,^{12–16} and more recently impregnating with silver acetate^{17–19} were introduced to enhance the grafts with bactericidal agents. Both are widely used. More recently, triclosan (5-chloro-2-(2,4-dichlorophenoxy)phenol) has been added to silver, and the combination of these two anti-infective agents has demonstrated promising results²⁰ in an in vitro study of grafts contaminated with a *Staphylococcus aureus* (ATCC 33591) obtained from the American Type Culture Collection (Manassas, VA, US). To challenge this latest generation antimicrobial graft, a similar experimental protocol was designed but a decision was taken to contaminate the grafts with bacteria and fungi directly collected from infected aortic grafts retrieved from patients. Indeed, since patients suffering from aortic graft infection are often exposed pre-operatively to antibiotics, it may be hypothesized that “in-patient bacteria” are probably more challenging than the ATCC collection micro-organisms with regards to the development of antimicrobial resistance. Using clinically relevant micro-organisms, the aim of this in vitro experimental study was to compare the antimicrobial efficacy of the silver impregnated collagen coated polyester vascular graft with an identical graft combining silver and triclosan.

MATERIAL AND METHODS

Setting and study period

The study was conducted between June and December 2014 in the R&D laboratory Aquitaine Microbiologie at the University of Bordeaux. One non-antimicrobial and two antimicrobial vascular grafts manufactured by Maquet (La Ciotat, France) were investigated: (a) a standard knitted non-antimicrobial collagen coated polyester (InterGard) vascular graft (IG) acted as a control; (b) a silver knitted collagen coated polyester (InterGard Silver) vascular graft (IGS), containing silver acetate alone; and (c) a silver and triclosan knitted collagen coated polyester (InterGard Synergy) vascular graft (IGSy), containing triclosan in addition to silver acetate.

Clinical strains

For the purpose of the study, four micro-organisms were tested, three of which belong to a collection of clinical

strains collected from a cohort of 80 patients treated in the vascular surgery unit for aortic graft infections.

The three clinical strains used in this study were chosen on the basis of their prevalence in the unit together with their clinical significance in retrieved infected aortic grafts:

- methicillin resistant *S. aureus* (MRSA)
- *Escherichia coli* producing extended spectrum beta-lactamase (ESBL-EC)
- *Candida albicans*.

The fourth strain was *Staphylococcus epidermidis* (ATCC 12228, American Type Culture Collection, Manassas, VA).

Microbiological assays

The in vitro antimicrobial efficacy of the different contaminated grafts were evaluated by time to kill assays over 24 hours according to the standard test guideline M26-A recommended by the Clinical and Laboratory Standards Institute (CLSI).²¹ Three specimens of each graft were aseptically cut into 4 mm diameter samples using a single use 4 mm biopsy punch (Kai Medical, Solingen, Germany). Each graft sample was then immersed in an Eppendorf tube containing the test micro-organism in 120 μL of a Muller–Hinton broth culture (BioMérieux, France). The density of the micro-organism target inoculum was set at 5.0×10^5 colony forming units (CFU)/mL, resulting in a micro-organism density of 4,773 per mm^2 of graft. A standard plate count on trypticase soy agar plates was performed to determine the initial population of the test organisms. All serial dilutions were made in Eugon LT (Biokar diagnostic) a standard recommended neutralizer. Eight repeated measures of each graft for the four different micro-organisms at intervals 1, 4, 8, and 24 hours after incubation at 37 °C were collected. After each incubation time, 100 μL of the broth culture was subjected to serial dilutions to determine the concentration of the micro-organisms in the broth.

Sonication of the antimicrobial grafts

Sonication of the IGS and the IGSy grafts was performed to verify there were no residual micro-organisms on the graft surface. This was done in order to liberate and collect any viable micro-organisms potentially adhering to a graft. Graft samples were taken out of the inoculum broth culture and sonicated for 5 minutes at 48 Hz in a 1 mL fresh broth culture; 100 μL of the sonicated broth was spread onto a fresh agar plate and incubated for 18 hours at 37 °C. The absence of colonies on the plates following incubation would be further evidence of the antimicrobial action of the graft.

Statistical analysis

Statistical analysis was performed with GraphPad Prism V (GraphPad Software, Inc., San Diego, CA, USA). To determine the required number of repeated measurements for each graft at each of the interval times the same power calculation was used as reported by Ricco et al.²⁰ To obtain a study power

Table 1. Growth of *Staphylococcus epidermidis* is shown for the three vascular grafts (data are shown in CFU/mL and converted in \log_{10}). The two antimicrobial grafts (IGS and IGSy) are compared with the one non-antimicrobial graft (IG) by using the \log_{10} reduction factor (RF).

Variable	1 hour		4 hours		8 hours		24 hours	
	Mean (SE)	\log_{10}	Mean (SE)	\log_{10}	Mean (SE)	\log_{10}	Mean (SE)	\log_{10}
IG	3.13×10^5 (1.19×10^5)	5.50	3.87×10^6 (1.96×10^6)	6.59	4.05×10^6 (1.91×10^6)	6.61	3.05×10^6 (1.57×10^6)	6.48
IGS	1.20×10^5 (2.79×10^4)	5.08	5.98×10^4 (1.82×10^4)	4.78	3.02×10^4 (1.43×10^4)	4.48	1.81×10^3 (1.11×10^3)	3.26
IGSy	1.00×10^5 (2.32×10^4)	5.00	2.19×10^4 (1.03×10^4)	4.34	5.81×10^3 (5.24×10^3)	3.76	2.60×10^2 (1.68×10^2)	2.41

Variable	1 hour	4 hours	8 hours	24 hours
	\log_{10} RF	\log_{10} RF	\log_{10} RF	\log_{10} RF
IGS vs. IG	0.42	1.81	2.13	3.23*
IGSy vs. IG	0.50	2.25	2.84	4.07*

IG = Intergard; IGS = Intergard Silver; IGSy = Intergard Synergy combining silver and triclosan; SE = standard error.

An asterisk (*) represents bactericidal efficacy at each exposure time as defined by approved guideline Clinical and Laboratory Institute Standards M-26A, 1999.

of at least 80%, a six fold repeated measure of each time point was necessary. Therefore, all assays were repeated eight times; the lowest and highest measurements were eliminated and the six remaining measurements were analyzed.

The numbers of organisms were averaged as the mean CFU/mL. The averaged means were then transformed and expressed as mean \log_{10} CFU/mL. To present the data in the format of a "time to kill curve," the obtained viable mean \log_{10} counts (CFU/mL) at each investigated time point were plotted for each graft against time. Following the approved guideline CLSI M-26A, bactericidal activity was defined as a 3 \log_{10} reduction in CFU/mL, and

bacteriostatic activity was defined as a < 3 \log_{10} reduction in CFU/mL. The \log_{10} reduction factor (logRF) was calculated as \log_{10} of the non-antimicrobial graft (IG) minus \log_{10} of the respective antimicrobial grafts (IGS or IGSy) at each time point.

To determine a statistically significant difference between the mean \log_{10} CFU/mL counts of two antimicrobial vascular grafts at 4, 8, and 24 hours time points, a non-parametric Wilcoxon signed-rank test was calculated. To calculate one graft's overall difference in the mean \log_{10} CFU/mL over the four tested time points, a one-way analysis of variance (ANOVA) with a Bonferroni correction

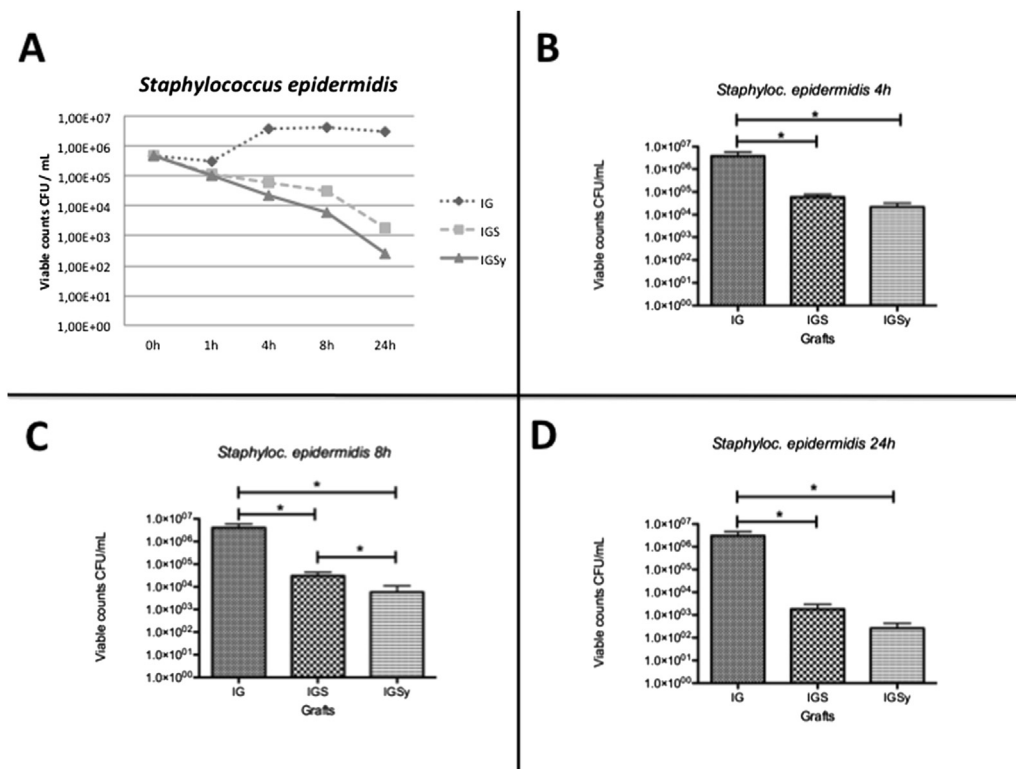


Figure 1. (A) Time to kill curves of three the vascular grafts contaminated by *Staphylococcus epidermidis*. (B, C, D) show the mean CFU/mL counts at 4, 8, and 24 hours, respectively. Non-parametric analysis using a Wilcoxon signed-rank test is used; asterisk (*) represents $p < .05$ significant difference between two grafts.

Table 2. Growth of methicillin resistant *Staphylococcus aureus* is shown for the three vascular grafts (data are shown in CFU/mL and converted in \log_{10}), the two antimicrobial grafts (IGS and IGSy) are compared with the one non-antimicrobial graft (IG) by using the \log_{10} reduction factor (RF).

Variable	1 hour		4 hours		8 hours		24 hours	
	Mean (SE)	\log_{10}	Mean (SE)	\log_{10}	Mean (SE)	\log_{10}	Mean (SE)	\log_{10}
IG	1.18×10^6 (7.76×10^5)	6.07	4.25×10^6 (1.23×10^6)	6.63	3.50×10^6 (5.00×10^5)	6.54	5.17×10^6 (1.67×10^6)	6.71
IGS	2.88×10^5 (1.25×10^5)	5.46	2.16×10^5 (1.17×10^5)	5.33	1.95×10^5 (1.15×10^5)	5.29	2.50×10^4 (2.10×10^4)	4.40
IGSy	2.57×10^5 (1.10×10^5)	5.41	6.08×10^4 (5.58×10^4)	4.78	3.95×10^4 (2.92×10^4)	4.60	4.30×10^2 (2.13×10^2)	2.63

Variable	1 hour \log_{10} RF	4 hours \log_{10} RF	8 hours \log_{10} RF	24 hours \log_{10} RF
IGS vs. IG	0.61	1.29	1.26	2.32
IGSy vs. IG	0.66	1.84	1.95	4.08*

IG = Intergard; IGS = Intergard Silver; IGSy = Intergard Synergy combining silver and triclosan; SE = standard error.

An asterisk (*) represents bactericidal efficacy at each exposure time as defined by approved guideline Clinical and Laboratory Institute Standards M-26A, 1999.

was separately calculated for each graft. For all statistical tests, a value of $p < .05$ was considered statistically significant.

RESULTS

Sonication was performed to verify that there were no remaining micro-organisms on the graft when the IGS and the IGSy grafts proved to have bactericidal or fungicidal activity. Indeed, this was demonstrated by removing the graft sample from the broth culture and sonicating as described above. For the four tested micro-organisms, no

residual viable micro-organisms were found on the either the IGS or the IGSy sonicated grafts.

Vascular grafts and *S. epidermidis*

The mean CFU/mL, together with standard error and the corresponding \log_{10} transformations of the time to kill study, are summarized in Table 1 and plotted as time to kill curves in Fig. 1A. The non-antimicrobial IG graft showed a non-significant increase in the number of viable organisms after the fourth hour ($p = .19$ one-way ANOVA). During the same time, the antimicrobial vascular grafts showed a

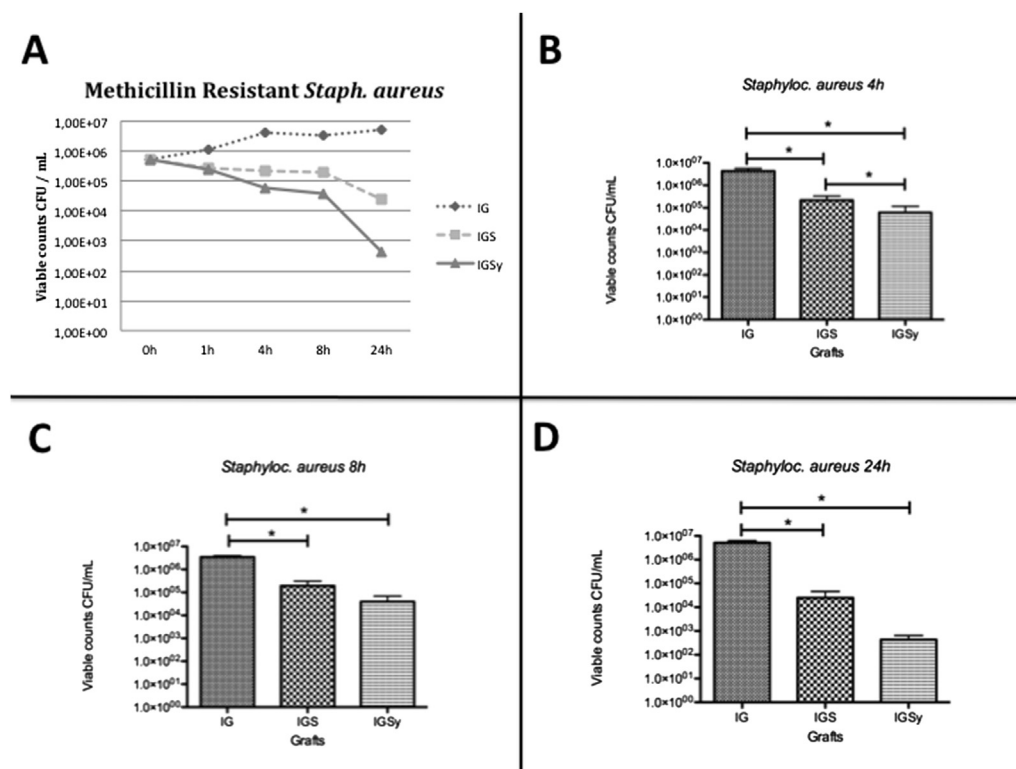


Figure 2. (A) Time to kill curves of the three vascular grafts contaminated by methicillin resistant *Staphylococcus aureus*. (B, C, D) The mean CFU/mL counts at 4, 8, and 24 hours, respectively. Non-parametric analysis using a Wilcoxon signed-rank test is used; asterisk (*) represents a $p < .05$ significant difference between two grafts.

Table 3. Growth of ESBL *Escherichia coli* is shown for the three vascular grafts (data are shown in CFU/mL and converted in log₁₀), the two antimicrobial grafts (IGS and IGSy) are compared with the one non-antimicrobial graft (IG) by using the log₁₀ reduction factor (RF).

Variable	1 hour		4 hours		8 hours		24 hours	
	Mean (SE)	Log ₁₀	Mean (SE)	Log ₁₀	Mean (SE)	Log ₁₀	Mean (SE)	Log ₁₀
IG	8.83 × 10 ⁵ (6.28 × 10 ⁵)	5.95	4.03 × 10 ⁶ (1.88 × 10 ⁶)	6.61	5.00 × 10 ⁶ (1.67 × 10 ⁶)	6.70	5.67 × 10 ⁶ (1.66 × 10 ⁶)	6.75
IGS	5.19 × 10 ⁴ (3.36 × 10 ⁴)	4.72	8.73 × 10 ³ (6.44 × 10 ³)	3.94	1.73 × 10 ⁴ (1.65 × 10 ⁴)	4.24	1.76 × 10 ⁴ (9.25 × 10 ³)	4.25
IGSy	2.43 × 10 ⁴ (1.64 × 10 ⁴)	4.39	8.46 × 10 ³ (6.43 × 10 ³)	3.93	2.21 × 10 ³ (1.58 × 10 ³)	3.34	9.49 × 10 ² (8.11 × 10 ²)	2.98

Variable	1 hour	4 hours	8 hours	24 hours
	Log ₁₀ RF	Log ₁₀ RF	Log ₁₀ RF	Log ₁₀ RF
IGS vs. IG	1.23	2.66	2.46	2.51
IGSy vs. IG	1.56	2.68	3.35*	3.78*

IG = Intergard; IGS = Intergard Silver; IGSy = Intergard Synergy combining silver and triclosan; SE = standard error. An asterisk (*) represents bactericidal efficacy at each exposure time as defined by approved guideline Clinical and Laboratory Institute Standards M-26A, 1999.

significant time dependent decrease in viable counts. Indeed for the *S. epidermidis* inoculated samples, the IGS and IGSy grafts both yielded significantly lower mean CFU/mL counts at 4, 8, and 24 hours ($p < .05$ Wilcoxon rank-sum test) than IG control graft (Fig. 1B–D). Both achieved > 3 logRF (Table 1) and fulfilled the efficacy criterion for bactericidal activity at 24 hours; yet they performed differently in their speed of antimicrobial action. For the IGS graft, one-way ANOVA showed a significant difference over time ($p = .002$; $F = 5.07$; $df = 4$) with a significance between all time points from 0 to 24 hours; for the IGSy graft, the difference was also significant ($p < .001$; $F = 6.3$;

$df = 4$; one-way ANOVA) with significant differences between all time points. Comparing the two antimicrobial vascular grafts, IGSy yielded significantly lower mean CFU/mL counts at 8 hours ($p = .03$ Wilcoxon rank-sum test) than IGS (Fig. 1C).

Vascular grafts and methicillin resistant *S. aureus*

The mean CFU/mL, together with standard error and the corresponding log₁₀ transformations of the time to kill study, are summarized in Table 2 and plotted as time to kill curves in Fig. 2A. The non-antimicrobial IG graft showed a significant increase in the number of viable organisms after

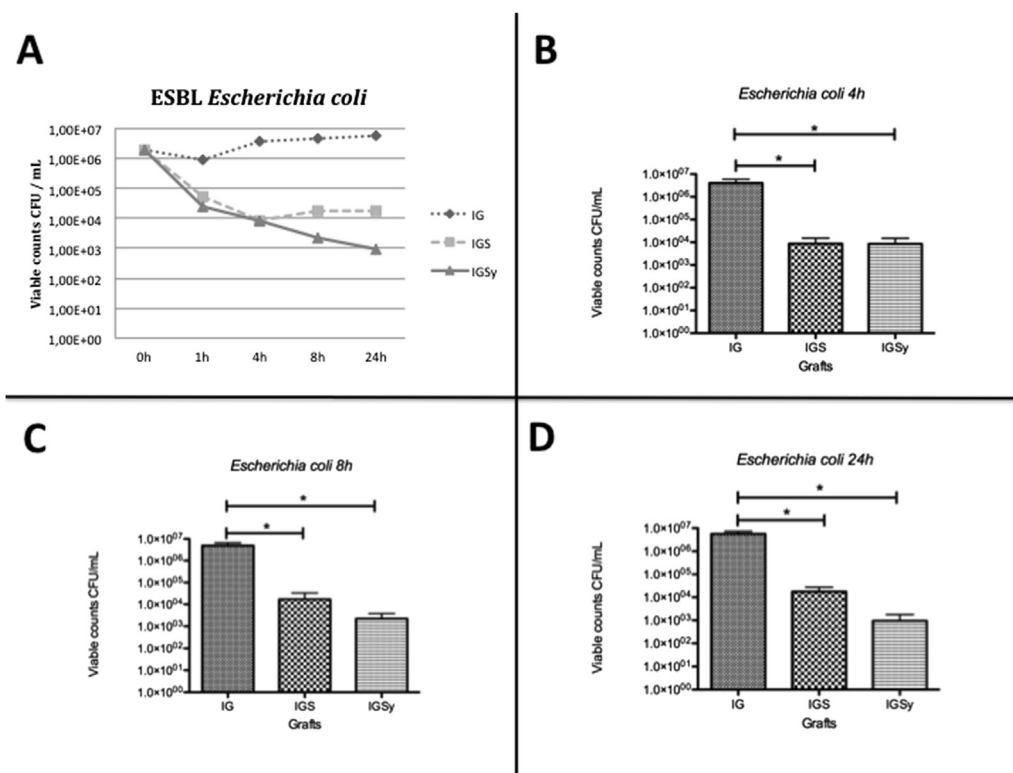


Figure 3. (A) Time to kill curves of the three grafts contaminated by EBSL *Escherichia coli*. (B, C, and D) The mean CFU/mL counts at 4, 8, and 24 hours, respectively. Non-parametric analysis using a Wilcoxon signed-rank test is used; asterisk (*) represents $p < .05$ significant difference between two grafts.

24 hours ($p = .003$; $F = 5.2$; $df = 4$; one-way ANOVA). The IGS and IGSy grafts yielded significantly lower mean CFU/mL counts at 4, 8, and 24 hours ($p < .05$ Wilcoxon rank-sum test) than IG control graft for the MRSA inoculated samples (Fig. 2B–D). Only the IGSy graft achieved > 3 logRF (Table 2) and fulfilled the efficacy criterion for bactericidal activity at 24 hours. For the IGS graft, one-way ANOVA showed no significant difference over time ($p = .058$; $F = 2.6$; $df = 4$); for the IGSy graft, the difference was significant ($p < .001$; $F = 6.1$; $df = 4$; one-way ANOVA) with significant differences between all time points. Comparing the two antimicrobial vascular grafts, IGSy yielded significantly lower mean CFU/mL counts at 4 hours ($p = .03$ Wilcoxon rank-sum test) than IGS (Fig. 2B).

Vascular grafts and ESBL-EC

The mean CFU/mL, together with standard error and the corresponding \log_{10} transformations of the time to kill study, are summarized in Table 3 and plotted as time to kill curves in Fig. 3A. The non-antimicrobial IG graft showed a significant increase in the number of viable organisms after 24 hours ($p = .003$; $F = 5.2$; $df = 4$; one-way ANOVA). The IGS and IGSy grafts yielded significantly lower mean CFU/mL counts at 4, 8, and 24 hours ($p < .05$ Wilcoxon rank-sum test) than IG control graft for the ESBL-EC inoculated samples (Fig. 3B–D). Only the IGSy graft achieved > 3 logRF (Table 3) at 8 and 24 hours and fulfilled the efficacy criterion for bactericidal activity. For the IGS graft, one-way ANOVA showed no significant difference over time ($p = .058$; $F = 2.6$; $df = 4$); for the IGSy graft, the difference was significant ($p = .001$; $F = 6.2$; $df = 4$; one-way ANOVA) with significant differences between all time points.

Vascular grafts and *C. albicans*

The mean CFU/mL, together with standard error and the corresponding \log_{10} transformations of the time to kill study, are summarized in Table 4 and plotted as time to kill curves in Fig. 4A. The non-antimicrobial IG graft showed a significant increase in the number of viable organisms after

24 hours ($p = .04$; $F = 5.9$; $df = 4$; one-way ANOVA). The IGS and IGSy grafts yielded significantly lower mean CFU/mL counts at 8 and 24 hours ($p < .05$ Wilcoxon rank-sum test) than IG control graft for the *C. albicans* inoculated samples (Fig. 4C,D) but only IGSy yielded significantly lower mean CFU/mL counts at 4 hours (Fig. 4B). The antimicrobial vascular grafts both achieved > 3 logRF (Table 4) and fulfilled the efficacy criterion for bactericidal activity at 24 hours; yet they performed differently in their antimicrobial action. For the IGS graft, one-way ANOVA showed no significant difference over time ($p = .09$; $F = 2.2$; $df = 4$); for the IGSy graft, the difference was significant ($p = .007$; $F = 2.1$; $df = 4$; one-way ANOVA) between all time points.

DISCUSSION

Using an in vitro experimental protocol previously described,²⁰ this study suggests that vascular grafts containing triclosan in addition to silver acetate (Intergard Synergy; IGSy) provide enhanced antimicrobial properties compared with grafts containing only silver acetate (Intergard Silver; IGS). This advantage was demonstrated when the grafts were challenged with either an ESBL-EC or a MRSA, since only the IGSy graft achieved > 3 logRF and fulfilled the efficacy criterion for bactericidal activity at 24 hours. For the fungus *C. albicans*, although both antimicrobial grafts achieved 24 hours of bactericidal activity, one-way ANOVA analysis demonstrated that the IGSy graft performed differently in terms of speed of action, appearing more active as early as 4 hours following graft inoculation. This is the first time that in vitro experiments report results in vascular graft infection with yeasts. Fungi are frequently implicated in aortic graft infections complicated by secondary aorto-enteric fistulae, ranging from 28%¹⁸ to 42%.²² Despite this frequent occurrence, only case reports have described fungal infection in vascular grafts. When prosthetic grafting is planned, the use of an IGSy graft in combination with antifungal therapy could represent a promising strategy to potentially reduce the risk of fungus infection or fungal contamination.

Table 4. Growth of *Candida albicans* is shown for the three vascular grafts (data are shown in CFU/mL and converted to \log_{10}). The two antimicrobial grafts (IGS and IGSy) are compared with the one non-antimicrobial graft (IG) by using the \log_{10} reduction factor (RF).

Variable	1 hour		4 hours		8 hours		24 hours	
	Mean (SE)	Log ₁₀	Mean (SE)	Log ₁₀	Mean (SE)	Log ₁₀	Mean (SE)	Log ₁₀
IG	1.01×10^5 (6.03×10^4)	5.01	3.38×10^5 (1.36×10^5)	5.53	7.88×10^5 (3.94×10^5)	5.90	7.88×10^5 (4.5×10^5)	5.90
IGS	7.12×10^4 (3.95×10^4)	4.85	1.34×10^5 (6.58×10^4)	5.13	6.18×10^4 (3.62×10^4)	4.79	2.45×10^2 (1.51×10^2)	2.39
IGSy	4.22×10^4 (1.74×10^4)	4.62	1.25×10^4 (7.24×10^3)	4.10	9.13×10^2 (6.3×10^2)	2.96	9.33×10^1 (2.1)	1.97
Variable	1 hour		4 hours		8 hours		24 hours	
	Log ₁₀ RF		Log ₁₀ RF		Log ₁₀ RF		Log ₁₀ RF	
IGS vs. IG	0.15		0.40		1.11		3.51*	
IGSy vs. IG	0.38		1.43		2.94		3.93*	

IG = Intergard; IGS = Intergard Silver; IGSy = Intergard Synergy combining silver and triclosan; SE = standard error.

An asterisk (*) represents bactericidal efficacy at each exposure time as defined by approved guideline Clinical and Laboratory Institute Standards M-26A, 1999.

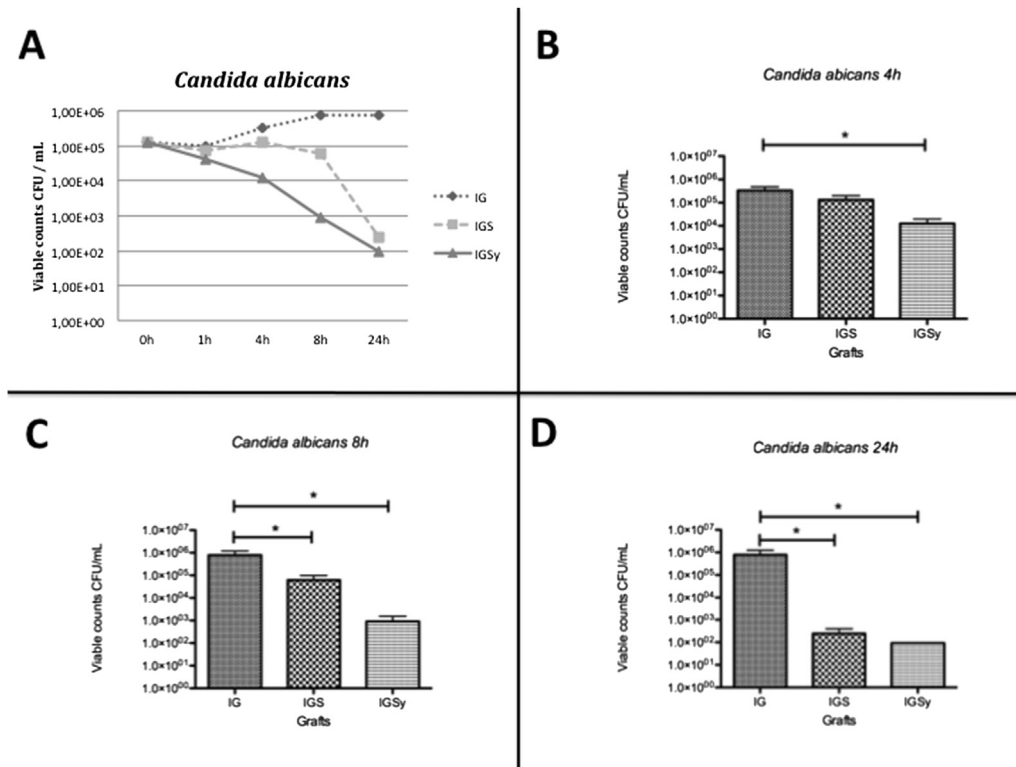


Figure 4. (A) Time to kill curves of the three grafts contaminated by the fungus *Candida albicans*. (B, C, D) show the mean CFU/mL counts at 4, 8, and 24 hours, respectively. Non-parametric analysis using a Wilcoxon signed-rank test is used; asterisk (*) represents $p < .05$ significant difference between two grafts.

Ricco et al.²⁰ experimenting with ATCC 33591 MRSA infection using the same three grafts demonstrated that the two antimicrobial grafts showed bactericidal activity against MRSA in vitro. They concluded that the IGSy graft showed antimicrobial efficacy after 8 hours compared with the IGS graft, which exhibited its antimicrobial properties after 24 hours. Regarding the MRSA experiments reported here, the results appear to be different. Indeed, the IGS failed to obtain a > 3 logRF after 24 hours. Two parameters may explain this discrepancy regarding the IGS graft. Firstly, the initial inoculum in this study was 4,773 per mm^2 , 1.9 fold more concentrated than in Ricco's setting (2,500 per mm^2). The aim was to explore the bactericidal behavior of the grafts in the situation of an infection rather than a contamination, explaining the choice for the initial inoculum concentration. Secondly, the MRSA strain was obtained from a clinical origin, directly harvested from infected aortic grafts explanted from patients suffering from abdominal vascular graft infection. In the frequent situation of a polymicrobial infection involving simultaneously Gram negative (such as ESBL-EC) and Gram positive bacteria, and fungi such as are found in secondary aorto-enteric fistulae,¹⁸ the use of an IGSy graft may be considered a safer option.

Nevertheless, this study has some limitations. Firstly, the study was limited to the first 24 hours following graft material inoculation although it is known that the first 30 days remain critical in patients operated on for an aortic graft infection.² However, thanks to the prompt recovery of the micro-organisms by mean of sonication²³ combined with a

rapid and appropriate antibiotic prescribing strategy based on a novel E-test approach,²⁴ it can be considered that beyond 24 hours after grafting, targeted antibiotic treatment can be administered to the patient. Indeed, during the first 24 hour post-procedure period, the cause of the graft infection remains unknown and it is hoped that it can be prevented by administering wide spectrum non-targeted antibiotics and the use of bactericidal grafts. Nevertheless, extending the protocol to at least a 7 day period could provide useful information regarding whether the effects of triclosan persist in patients who remain in an intensive care unit exposed to a variety of secondary contamination through respiratory or catheter origins. Secondly, many surgeons no longer use the Dacron IG graft alone but instead the graft is soaked with rifampicin.^{13–15} Future experiments should take into account this practice and compare the new antimicrobial grafts (IG or IGSy) with a rifampicin soaked graft as a control. Thirdly, by choosing "in patient bacteria" rather than standardized bacteria strains from the ATCC collection the reproducibility of the experimental study is affected. The results of this study are preliminary and based on a rather small collection of isolates. Further, bigger scale studies are warranted to evaluate the antimicrobial activity of the grafts on a more representative collection of clinical isolates. However, regarding MRSA, the original protocol confirmed the positive results of triclosan as reported by Ricco et al.¹⁹ when using MRSA obtained from the ATCC collection. Fourthly, as an in vitro experiment, the antimicrobial activities of silver ions and triclosan

in a biological environment remain unexplored. Animal *in vivo* experimental models^{25,26} could be helpful to further elucidate the bio-availability of these antiseptic agents and confirm their promising *in vitro* anti-infectious properties.

In conclusion, in the *in vitro* conditions studied, the Synergy vascular graft combining silver with triclosan demonstrated better short-term antimicrobial activity than the silver graft for all micro-organisms tested.

CONFLICT OF INTEREST

None.

FUNDING

This experimental study was conducted at the University of Bordeaux R&D laboratory which has received funding through a research grant supplied by MAQUET. The grant was used solely to compensate a full time technician and for time and materials to conduct the experiments. No physicians received direct honoraria from this grant. X.B. received speaker fees from MAQUET to present the preliminary results of this study during the Charing Cross meeting held in London in April 2015.

REFERENCES

- Seeger JM. Management of patients with prosthetic vascular graft infection. *Am Surg* 2000;**66**:166–77.
- Garot M, Delannoy P-Y, Meybeck A, Sarraz-Bournet B, d' Elia P, d' Escrivan T, et al. Intra-abdominal aortic graft infection: prognostic factors associated with in hospital mortality. *BMC Infect Dis* 2014;**14**:215.
- Berger P, Van Herwaarden JA, Harkisoen S, De Vries JPM, Ekkelenkamp M, Moll FL. Surgical treatment of infected aortic grafts. *J Cardiovasc Surg (Torino)* 2012;**53**:719–34.
- Daenens K, Fourneau I, Nevelsteen A. Ten-year experience in autogenous reconstruction with the femoral vein in the treatment of aortofemoral prosthetic infection. *Eur J Vasc Endovasc Surg* 2003;**25**:240–5.
- Brown KE, Heyer K, Rodriguez H, Eskandari MK, Pearce WH, Morasch MD. Arterial reconstruction with cryopreserved human allografts in the setting of infection: a single center experience with midterm follow up. *J Vasc Surg* 2009;**49**:660–6.
- O'Connor S, Andrew P, Batt M, Becquemin JP. A systematic review and meta-analysis of treatments for aortic graft infection. *J Vasc Surg* 2006;**44**:38–45.
- Harlander-Locke MP, Harmon LK, Lawrence PF, Oderich GS, McCready RA, Morasch MD, et al. The use of cryopreserved aortoiliac allograft for aortic reconstruction in the United States. *J Vasc Surg* 2014;**59**:669–74.
- Kieffer E, Gomes D, Chiche L, Fléron M-H, Koskas F, Bahnini A. Allograft replacement for infrarenal aortic graft infection: early and late results in 179 patients. *J Vasc Surg* 2004;**39**:1009–17.
- Vogt PR. Arterial allografts in treating aortic graft infections: something old, something new. *Semin Vasc Surg* 2011;**24**:227–33.
- Brahmanandam S, Clair D, Bena J, Sarac T. Adjunctive use of the superficial femoral vein for vascular reconstructions. *J Vasc Surg* 2012;**55**:1355–62.
- Dorweiler B, Neufang A, Chaban R, Reinstadler J, Duenschede F, Vahl C-F. Use and durability of femoral vein for autologous reconstruction with infection of the aortoiliac-femoral axis. *J Vasc Surg* 2014;**59**:675–83.
- Schneider F, O'Connor S, Becquemin JP. Efficacy of collagen silver-coated polyester and rifampin-soaked vascular grafts to resist infection from MRSA and *Escherichia coli* in a dog model. *Ann Vasc Surg* 2008;**22**:815–21.
- Goëau-Brissonnière O, Javerliat I, Koskas F, Coggia M, Pechère J-C. Rifampin-bonded vascular grafts and post-operative infections. *Ann Vasc Surg* 2011;**25**:134–42.
- Bandyk DF, Novotney ML, Johnson BL, Back MR, Roth SR. Use of rifampin-soaked gelatin-sealed polyester grafts for *in situ* treatment of primary aortic and vascular prosthetic infections. *J Surg Res* 2001;**95**:44–9.
- Oderich GS, Bower TC, Hofer J, Kalra M, Duncan AA, Wilson JW, et al. *In situ* rifampin-soaked grafts with omental coverage and antibiotic suppression are durable with low reinfection rates in patients with aortic graft enteric erosion or fistula. *J Vasc Surg* 2011;**53**:99–106. 107.e1–7; discussion 106–7.
- Hayes PD, Nasim A, London NJ, Sayers RD, Barrie WW, Bell PR, et al. *In situ* replacement of infected aortic grafts with rifampicin-bonded prostheses: the Leicester experience (1992 to 1998). *J Vasc Surg* 1999;**30**:92–8.
- Batt M, Jean-Baptiste E, O'Connor S, Bouillanne P-J, Haudebourg P, Hassen-Khodja R, et al. *In-situ* revascularisation for patients with aortic graft infection: a single centre experience with silver coated polyester grafts. *Eur J Vasc Endovasc Surg* 2008;**36**:182–8.
- Delva JC, Déglise S, Bérard X, Dubuisson V, Delva F, Stecken L, et al. *In-situ* revascularisation for secondary aorto-enteric fistulae: the success of silver-coated Dacron is closely linked to a suitable bowel repair. *Eur J Vasc Endovasc Surg* 2012;**44**:417–24.
- Ricco J-B, Assadian O. Antimicrobial silver grafts for prevention and treatment of vascular graft infection. *Semin Vasc Surg* 2011;**24**:234–41.
- Ricco J-B, Assadian A, Schneider F, Assadian O. *In vitro* evaluation of the antimicrobial efficacy of a new silver-triclosan vs a silver collagen-coated polyester vascular graft against methicillin resistant *Staphylococcus aureus*. *J Vasc Surg* 2012;**55**:823–9.
- National Committee for Clinical Laboratory Standards (NCCLS). *Methods for determining bactericidal activity of antimicrobial agents*. Approved guideline (M26-A). Wayne, Pennsylvania. 1999.
- Batt M, Jean-Baptiste E, O'Connor S, Saint-Lebes B, Feugier P, Patra P, et al. Early and late results of contemporary management of 37 secondary aortoenteric fistulae. *Eur J Vasc Endovasc Surg* 2011;**41**:748–57.
- Berard X, Pinaquy J-B, Stecken L, Wille H, Deglise S, Ducasse E, et al. Use of 18F-fluorodeoxyglucose positron emission tomography-computed tomography and sonication for detection of infection after peripheral stent fracture. *Circulation* 2014;**129**:2437–9.
- Jenkins SG, Schuetz AN. Current concepts in laboratory testing to guide antimicrobial therapy. *Mayo Clin Proc* 2012;**87**:290–308.
- Gao H, Sandermann J, Prag J, Lund L, Lindholt JS. Prevention of primary vascular graft infection with silver-coated polyester graft in a porcine model. *Eur J Endovasc Surg* 2010;**39**:472–7.
- Hernández-Richter T, Schardey HM, Wittmann F, Mayr S, Schmitt-Sody M, Blasenbren S, et al. Rifampin and triclosan but not silver is effective in preventing bacterial infection of vascular Dacron graft material. *Eur J Vasc Endovasc Surg* 2003;**26**:550–7.