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In vitro Evidence of Improved Antimicrobial Efficacy of Silver and Triclosan Containing Vascular Grafts Compared with Rifampicin Soaked Grafts

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WHAT THIS PAPER ADDS

Thanks to efficient bactericidal activity against Gram positive and negative bacteria as well as yeasts, this study provides in vitro evidence in favour of a vascular graft containing silver and triclosan over a rifampicin soaked graft. The study confirms that rifampicin soaking exposes patients to the risk of hosting rifampicin resistant bacteria compromising the future efficacy of rifampicin.

Objectives: The aim was to compare the antimicrobial efficacy of four different grafts: a standard graft (Intergard, IG), an IG graft soaked in rifampicin (IGrif), a silver impregnated graft (Intergard Silver, IGS), and a silver + triclosan impregnated graft (Intergard Synergy, IGSy).

Methods: This was a seven day in vitro study. The IG, IGrif, IGS, and IGSy grafts were each contaminated separately with the following microorganisms: *Staphylococcus epidermidis,* Methicillin resistant *Staphylococcus aureus* (MRSA), *Escherichia coli*, and *Candida albicans* from both clinical and American Type Culture Collection (ATCC) origins. The in vitro antimicrobial efficacy was evaluated by time to kill assays at T0, T24h, T48h, T72h, and T168h. Bactericidal activity was defined as >3 $\log_{10}$ reduction factor (logRF). Additionally, Rifampicin, triclosan and silver resistance development were screened.

Results: As anticipated for the non-antimicrobial IG, all microorganism strains proliferated. The IGSy and the IGS showed a seven day bactericidal efficacy (>3 logRF) for all tested microorganisms. This efficacy was confirmed at all time points for IGSy only, demonstrating faster bactericidal efficacy than IGS. The IGrif demonstrated a seven day bactericidal efficacy against the ATCC MRSA only, while showing no activity against *C. albicans* and ATCC *E. coli*. Regarding ATCC *S. epidermidis,* clinical MRSA and clinical *E. coli*, IGrif, although bactericidal at earlier time points, lost its antimicrobial efficacy at seven days leading to the emergence of rifampicin resistant mutants in four of six, two of six, and two of six assays, respectively. Mutant strains were also detected in ATCC MRSA in one of six assays. No triclosan or silver resistance has emerged at T7days.

Conclusion: For all microorganisms tested, the Synergy graft combining silver with triclosan demonstrated a more sustainable and efficient seven day antimicrobial activity than the rifampicin soaked graft. The emergence of rifampicin resistant mutants suggests preference for a Synergy graft over a graft soaked in rifampicin, to prevent or treat an infection when a biological solution is not feasible.

Keywords: Vascular graft, Infection, Triclosan, Silver, Rifampicin, Aortic surgery
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INTRODUCTION

Prosthetic reconstructions in an infected operative field in the absence or in the presence of contaminated (endo) vascular devices can be optimally performed when a biological solution is not feasible.[1] Silver and more recently silver and triclosan impregnated grafts have been used in

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this clinically challenging situation.[2] Using an in vitro experimental protocol previously described,[3] it was shown that vascular grafts containing triclosan in addition to silver acetate (Intergard Synergy, Maquet, Orleans, France; IGSy) provided enhanced antimicrobial properties compared with grafts containing silver acetate only (Intergard Silver; IGS).[4] This advantage was demonstrated when grafts were contaminated with either *Escherichia coli* or methicillin resistant *Staphylococcus aureus* (MRSA); only the IGSy graft fulfilled the efficacy criterion for bactericidal activity at 24 h. For fungus *Candida albicans*, while both antimicrobial grafts achieved a 24 h bactericidal activity, only the IGSy graft demonstrated its bactericidal efficacy as early as 4 h following graft inoculation.

However, two limitations led to questioning this experimental protocol:[1] Why limit the study period to 24 h since it is known that patients remain vulnerable under untargetted antibiotic therapy for longer periods pending the identification of the causative microorganism?[2] Why not compare the antimicrobial grafts with the commonly used rifampicin soaked grafts[5] use of which was recently contested because of the high risk of emergence of rifampicin resistant strains when used in monotherapy?[1]

Therefore, the aim of the present study was to compare, over a seven day period, the antimicrobial efficacy of four different grafts: a standard knitted collagen coated polyester vascular graft (Intergard, IG), the same IG graft soaked in rifampicin (IGrif) (5000 mg/L and 45,000 mg/L for 10 min), a silver impregnated collagen coated polyester vascular graft (Intergard Silver, IGS), and a silver + triclosan impregnated graft (Intergard Synergy, IGSy). Additionally, the frequency of emergence of rifampicin resistant mutants was evaluated.

METHODS

Setting and grafts

This seven day in vitro assessment is a follow up of a previous 24 h study published in the *European Journal of Vascular and Endovascular Surgery* in 2016.[4] The present study was conducted between September 2016 and May 2018 in the Research and Development laboratory Aquitaine Microbiologie at the University of Bordeaux. Three vascular grafts manufactured by GETINGE-MAQUET (La Ciotat, France) were investigated:

- a standard knitted non-antimicrobial collagen coated polyester (Intergard) vascular graft either soaked (IGrif) or not (IG) in rifampicin (5000 mg/L for 10 min) which was the routine protocol;
- a silver knitted collagen coated polyester (Intergard Silver) vascular graft (IGS), containing silver acetate alone;
- a silver triclosan knitted collagen coated polyester (Intergard Synergy) vascular graft (IGSy), containing triclosan in addition to silver acetate.

Specimens were obtained from the three different types of grafts (IG, IGS, and IGSy) and were aseptically cut into 4 mm diameter samples using a single use 4 mm biopsy punch (Kai Medical, Solingen, Germany). To avoid "intra-batch" differences, for each commercialised graft (IG, IGS and IGSy), three individual specimens originating from three different batches were pooled.

Strain collection

For the purpose of the study, four microorganisms were tested from both the American Type Culture Collection (ATCC, Manassas VA, USA) and from strains with clinical origins, except for *S. epidermidis*. The three clinical strains (MRSA, extended spectrum beta lactamase [ESBL] *E. coli*, and *C. albicans*) were taken from a collection recovered from a cohort of 80 patients treated in the vascular surgery unit for aortic graft infections. The strains were chosen on the basis of their prevalence in the unit together with their clinical significance in retrieved infected aortic grafts:

- *S. epidermidis* (ATCC 12228)
- Methicillin resistant *S. aureus* MRSA (ATCC 33591), and one MRSA from clinical origin
- *E. coli* (ATCC *K12* MG1655 rifampicin resistant), and one *E. coli* producing an ESBL from clinical origin
- *C. albicans* (ATCC 10231) and one isolate from clinical origin.

Microbiological assays

The in vitro antimicrobial efficacy of the different contaminated grafts were evaluated by time to kill assays at T0, T24h, T48h, T72h, and T168h (7 days) according to the standard test guideline M26-A recommended by the Clinical and Laboratory Standards Institute (CLSI).[6] The strain cultures were calibrated at an inoculum of 10^5 colony forming unit (CFU)/mL resulting in a microorganism density of 4773 per mm^2 of graft. Each graft sample was immersed in a 1.5 mL Eppendorf tube containing the test microorganism in 500 µL of a Muller—Hinton broth (BioMérieux, France) and incubated at 37 °C. An additional 500 µL of fresh broth was added to all tubes after 72 h of incubation to avoid microorganism starvation. Time slots for colony counting and molecular analysis of the isolates were set at 24 h, 48 h, 72 h, and 7 days and standard plate counts were performed on trypticase-soy agar plates. For this, at each incubation time, 100 µL of broth culture was subjected to serial dilutions to determine the concentration of the microorganisms in the broth. For each combination graft/microorganism, eight repeated measures were carried out. The lowest and highest measurements were eliminated and the six remaining measurements were analysed. Additionally, For MRSA, *C. albicans* and ESBL *E. coli*, both of clinical origin, the same microbiological protocol was reproduced using a higher concentration of rifampicin of 45,000 mg/L, as described previously.[5]

Sonication of the antimicrobial grafts

Sonication of the contaminated grafts was performed to check the absence of residual microorganisms on the graft

Table 1. Rifampicin minimum inhibitory concentrations (MICs) and molecular characterisation of the strains with *rpoB* mutations after 7 days

Strains	Rifampicin MIC of parental strains	*rpoB* mutations at seven days at 5000 mg/L rifampicin	*rpoB* mutations at seven days at 45,000 mg/L rifampicin
E. coli (rifampicin resistant), ATCC	>512 mg/L		
E. coli ESBL positive, clinical strain	4 mg/L	572 Isoleucine/Leucine	572 Isoleucine/Leucine
S. aureus MRSA, ATCC	0.1 mg/L	481 Histidine/Asparagine 471 Aspartate/Tyrosine	
S. aureus MRSA, clinical strain	4 mg/L	481 Histidine/Asparagine 468 Glycine/Lysine	–
S. epidermidis, ATCC	0.05 mg/L	471 Aspartate/Asparagine 481 Histidine/Tyrosine	
Candida albicans, ATCC	>512 mg/L		
Candida albicans, clinical strain	>512 mg/L		

ATCC = American type culture collection; MIC = minimum inhibitory concentration; MRSA = methicillin-resistant staphylococcus aureus; ESBL = extended-spectrum beta-lactamases; rpoB = gene encoding the beta subunit of ribonucleic acid (RNA) polymerase.

surface. Sonication was performed to liberate and collect any viable microorganisms potentially adhering to a graft. Graft samples were taken out of the broth culture and sonicated (Fisher scientific FB 15050, Germany) for 5 min at 48 Hz in 1 mL of fresh broth culture. Then 100 µL of the sonicated broth was spread onto a fresh agar plate and incubated for 18 h at 37 °C for bacterial isolates and for 48 h at 30 °C for *C. albicans*. The absence of colonies on agar plates following incubation would be further evidence of the antimicrobial action of the graft.

Screening for rifampicin, triclosan and silver resistance emergence

For rifampicin, conventional antibiograms were performed as recommended by the European Committee for Antibiotic Susceptibility Testing (EUCAST) and determination of

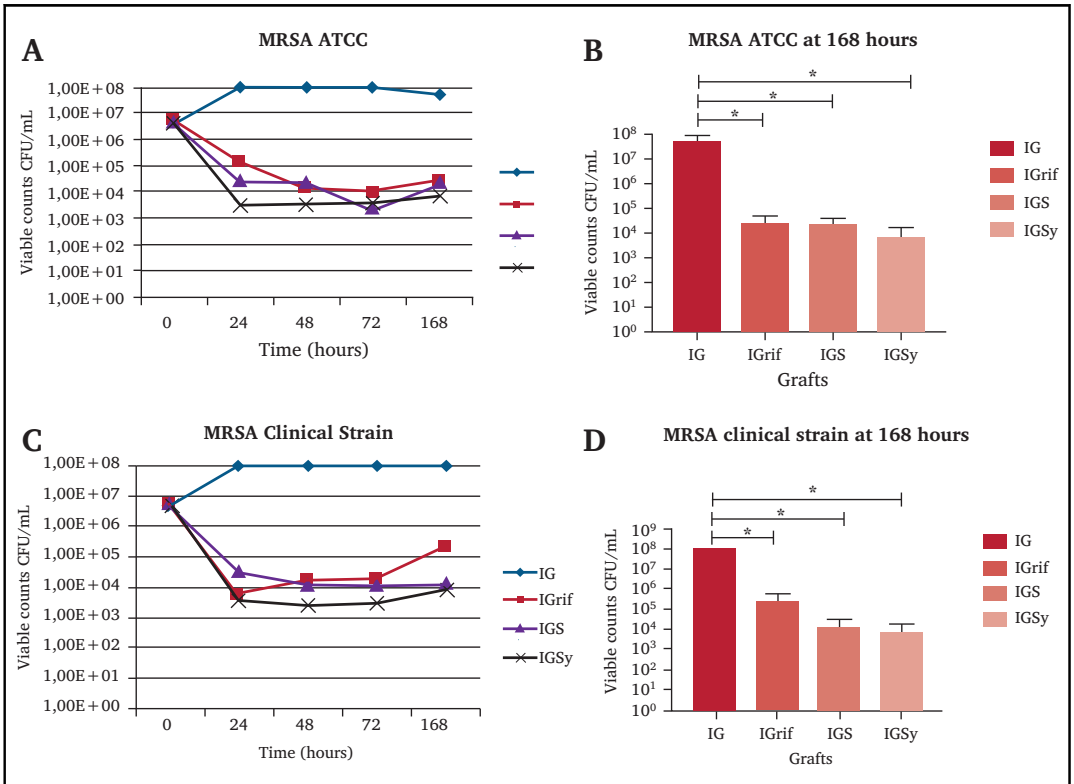

Figure 1. Time to kill curves (A, C) and Wilcoxon paired test at 168 h (B, D) of the four grafts contaminated by methicillin resistant *Staphylococcus aureus* (MRSA): American type culture collection (ATCC) (A, B) and clinical strain (C, D). CFU = colony forming unit; IG = Intergard; IGrif = Intergard rifampicin soaked (5000 mg/L); IGS = Intergard Silver; IGSy = Intergard Synergy.

rifampicin minimum inhibitory concentrations (MIC) were realised by agar dilution for all strains at baseline and at all time slots (Table 1). With regards to triclosan and silver, resistance development was sought for the strains exposed to IGS and IGSy by two approaches. The first one consisted of setting up an in house antibiogram for all parent and mutant strains using blank discs on which were placed 10 µL of solutions at 2 and 10 mg/L of triclosan or silver (10, 50, and 100 mg/L). Afterwards the inhibition zones were screened for any reduction in zone diameters at 7 days.

All strains showing a minimum twofold increase in rifampicin minimum inhibitory concentration (MIC) were screened at a molecular level for rifampicin resistance determinants.[7] Mutations in the beta subunit of the ribonucleic acid (RNA) polymerase gene (*rpoB)* known to be responsible for rifampicin resistance were sought by polymerase chain reaction (PCR) using a thermocycler (CFX96 Biorad, France) using previously described primers.[8–10] Triclosan[11] and silver[12] resistance determinants were also screened using the described primers. The resistance genes sought were *FabI*, *FabK*, *FabV*, *FabL*, *AcrB, and silA*). All PCR products were sent for nucleotide sequencing to a sequencing platform (Eurofin genomics, Anzinger, Germany). All sequences were analysed using BLAST Nucleotide Sequence Database (https://blast.ncbi.nlm.nih.gov)

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 as reported by Ricco et al.[3] was used. To obtain a study power of at least 80%, a sixfold repeated measure of each time point was necessary. All assays were repeated eight times, with the lowest and highest measures eliminated and the six remaining measures analysed. The numbers of microorganisms 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.[6] 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 (IGrif, IGS, and IGSy) at each time point. To determine a statistically significant difference between the mean $\log_{10}$ CFU/mL counts of the vascular grafts at interval time points, a non-parametric paired Wilcoxon signed rank test was calculated.

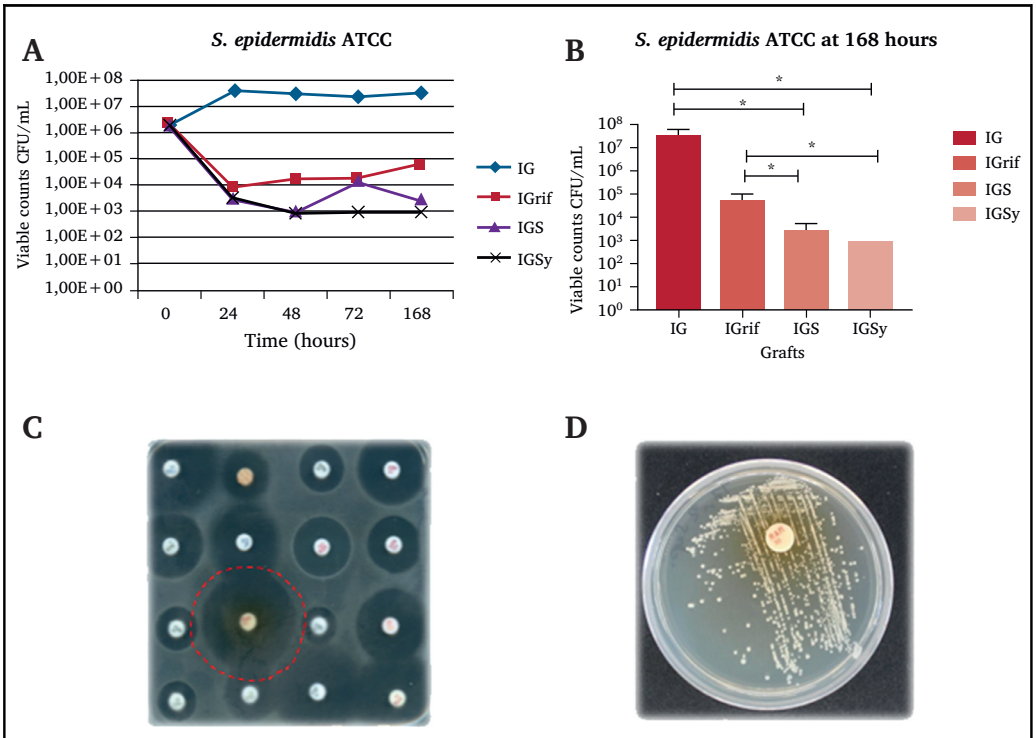

Figure 2. Time to kill curves (A) and Wilcoxon paired test at 168 h (B) of the four grafts contaminated *Staphylococcus epidermidis (S. epidermidis*) American type culture collection (ATCC). (C) EUCAST testing of 16 antibiotics, a red dotted line surrounds the rifampicin one with a MIC of 0.05 mg/L. (D) Resistance to rifampicin at seven days with a MIC > 512 mg/L. CFU = colony forming unit; IG = Intergard; IGrif = Intergard rifampicin soaked (5000 mg/L); IGS = Intergard Silver. IGSy = Intergard Synergy; MIC = minimum inhibitory concentration.

RESULTS

Non-antimicrobial grafts

As expected, IG grafts showed no antimicrobial activity on any of the strains (Figs. 1—4) and served as a control (Tables 2—4).

IGS silver grafts

Sonication of IGS graft samples did not provide additional viable strains.

Bactericidal efficacy was observed at seven days for all tested microorganisms. For both the MRSA strains (Fig. 1 and Table 2), *S. epidermidis* (Fig. 2 and Table 2) and ATCC *E. coli* (Fig. 3A and B and Table 3), this efficacy appeared as soon as 24 h following contamination. For clinical ESBL *E. coli* isolate (Fig. 3C and D and Table 3), antimicrobial activity was reached on the second day following contamination. For *C. albicans* from both origins, IGS demonstrated its efficacy only after 7 days (Fig. 4 and Table 4).

IGSy grafts combining silver and triclosan

Sonication of IGSy graft samples did not provide additional viable strains. As for the IGS graft, the IGSy demonstrated a seven day bactericidal efficacy for all tested microorganisms. However, this antimicrobial activity was effective as soon as 24 h for all strains (Figs. 1—4 and Tables 2—4). Screening for triclosan and silver resistant determinants by PCR showed no emergence of resistance to neither of the compounds in the seven day old cultures.

IGrif rifampicin soaked grafts

At a concentration of 5000 mg/L, IGrif demonstrated a seven day antimicrobial efficacy only against ATCC MRSA (Fig. 1A and B and Table 2). As expected, IGrif had no antimicrobial activity against *C. albicans* (Fig. 4 and Table 4) and ATCC *E. coli* (Fig. 3A and B and Table 3). For *S. epidermidis* (Fig. 2 and Table 2), clinical MRSA (Fig. 1C and D and Table 2), and clinical *E. coli* (Fig. 3C and D and Table 3), IGrif although bactericidal at earlier time points, gradually lost its antimicrobial efficacy over time. This was due to the emergence of rifampicin resistant mutants in four of six, two of six, and two of six assays, respectively. Mutants were also recovered in ATCC MRSA in one of six assays. When rifampicin was used at 5000 mg/L, at seven days *S. epidermidis*, ATCC MRSA, and clinical MRSA mutants exhibited a rifampicin MIC superior to 512 mg/L while the parent strains had a rifampicin MIC of 0.05, 0.1 and 4 mg/L respectively. The *E. coli* clinical rifampicin resistant mutants exhibited a rifampicin MIC of 256 mg/L while the parent strain had a MIC of 4 mg/L. By mean of a combination of PCR and nucleotide sequence analysis, the rifampicin resistant generated mutants showed the presence of rifampicin resistance associated mutations in the *rpo*B genes (Table 1). Moreover, when the

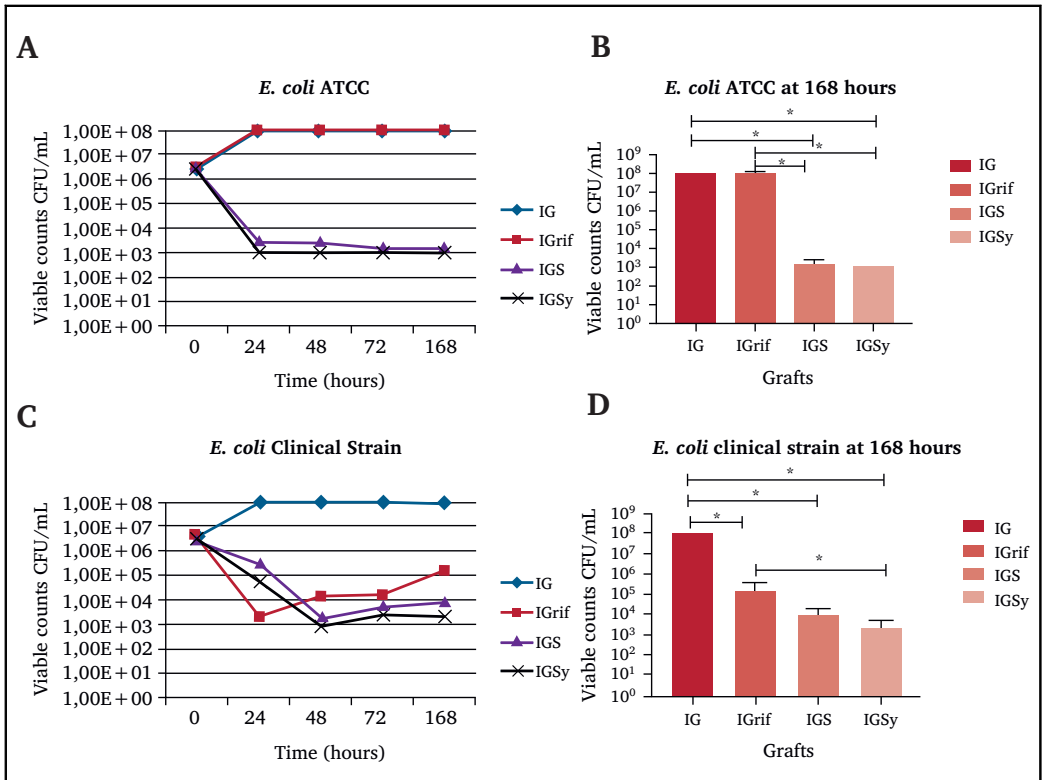

Figure 3. Time to kill curves (A,C) and Wilcoxon paired test at 168 h (B, D) of the four grafts contaminated by *Escherichia coli* (*E. coli*): American type culture collection (ATCC) (A, B) and clinical strain (C, D). CFU = colony forming unit; IG = Intergard; IGrif = Intergard rifampicin soaked (5000 mg/L); IGS = Intergard Silver. IGSy = Intergard Synergy.

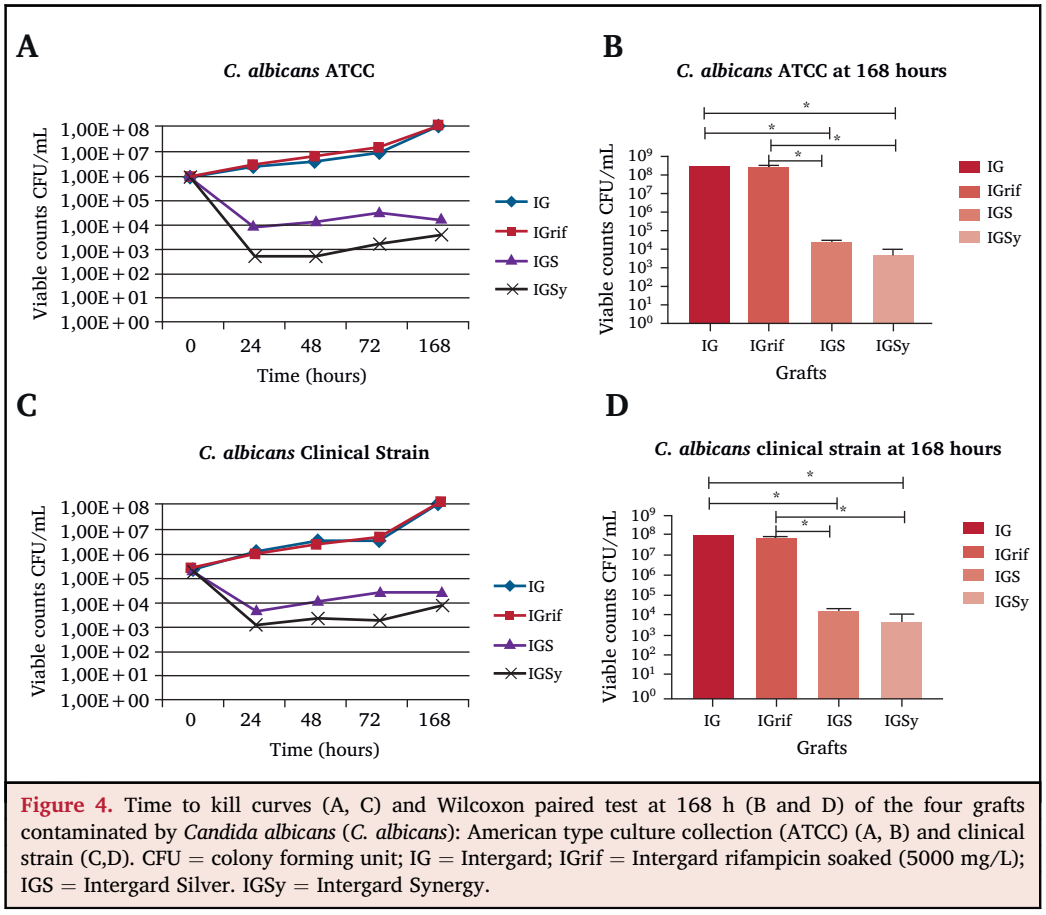

Figure 4. Time to kill curves (A, C) and Wilcoxon paired test at 168 h (B and D) of the four grafts contaminated by *Candida albicans* (*C. albicans*): American type culture collection (ATCC) (A, B) and clinical strain (C,D). CFU = colony forming unit; IG = Intergard; IGrif = Intergard rifampicin soaked (5000 mg/L); IGS = Intergard Silver. IGSy = Intergard Synergy.

rifampicin concentration was increased to 45,000 mg/L, the results were similar regarding clinical *E. coli* (Figs S5 and S6) and clinical *C. albicans* (data not shown), but were significantly improved regarding clinical MRSA as no rifampicin resistance was observed at T7 days (Figs S6 and S7).

DISCUSSION

In this in vitro study, for all tested microorganisms, the Synergy graft combining silver with triclosan demonstrated a more sustainable and efficient seven day antimicrobial activity compared with the soaked rifampicin grafts.

Because of promising animal experimentation results,[13–17] rifampicin soaking of collagen or gelatin coated knitted Dacron graft has been widely used to prevent[18,19] or to treat vascular graft infection.[20–22] However, several concerns arose from this practice. (1) How can this Gram positive cocci targetted monotherapy cover polymicrobial infections such as in secondary aorto-enteric fistulae associated with the frequent combination of Gram positive, Gram negative bacteria, and fungi in up to 40% of cases?[22,23] Indeed, the results clearly showed no efficacy against fungal infection, as expected since rifampicin has no activity on yeast. Regarding *E. coli* strains, because of the high concentration of rifampicin employed (5000 mg/L), rifampicin was effective against the *E. coli* clinical strain (initial MIC at 4 mg/L) at earlier time points, giving a false impression of efficacy[24] since this effect faded away overtime due to the emergence of resistant mutants. Exactly the same phenomenon was observed with the clinical strain of MRSA (initial MIC at 4 mg/L), confirming the ephemeral antimicrobial activity of rifampicin soaking. The "bombing strategy" by elevating rifampicin concentration from 5000 mg/L to 45,000 mg/L was only effective for clinical MRSA and did not show any improvement for clinical *E. coli and clinical C. albicans*.

(2) How great is the risk of the emergence of rifampicin resistance in patients treated with rifampicin soaked grafts, and how frequent is the prevalence of a vascular graft infection with a causative strain already rifampicin resistant? Indeed, in the study the clinical strain of MRSA collected from an infected graft was rifampicin resistant (initial MIC at 4 mg/L). It may be hypothesised that this scenario is favoured by a high prevalence of patients already treated by multiples antibiotics, promoting a higher risk of drug resistance. In routine clinical practice, rifampicin monotherapy is not recommended because of the high risk of generating drug resistant mutants.[25] Resistance to rifampicin involves mutations in the *rpoB* gene.[8–10] In this study, mutant strains resistant to rifampicin emerged after seven day exposure to the drug (contained in the IGrif grafts). Indeed, many studies have reported mutations in the rifampin resistance determining regions of *rpoB* gene of many bacterial species including *S. aureus*[10] *and S. epidermidis.*[9] The in vitro mutations observed were identical to

Table 2. Growth of MRSA and *S. epidermidis*.

	24 h		48 h		72 h		168 h	
	Mean	Log_{10}/ Log_{10} RF	Mean	Log_{10}/ Log_{10} RF	Mean	Log_{10}/ Log_{10} RF	Mean	Log_{10}/ Log_{10} RF
MRSA ATCC								
IG	9.2×10^7	7.96	1×10^8	8	1×10^8	8	1×10^8	8
IGrif	1.24×10^5	5.09	1.16×10^4	4.06	9×10^3	3.95	2.22×10^4	4.35
IGS	2.89×10^4	4.46	2.31×10^4	4.36	2.23×10^3	3.35	2.07×10^4	4.32
IGSy	2.91×10^3	3.46	3.56×10^3	3.55	3.73×10^3	3.57	6.99×10^3	3.84
IGrif vs. IG		2.87[b]		3.94[a,b]		4.05[a,b]		3.31[a,b]
IGS vs. IG		3.5[a,b]		3.64[a,b]		4.65[a,b]		3.34[a,b]
IGSy vs. IG		4.5[a,b]		4.45[a,b]		4.43[a,b]		3.81[a,b]
MRSA clinical strain								
IG	1×10^8	8	1×10^8	8	1×10^8	8	1×10^8	8
IGrif	4.98×10^3	3.7	1.6×10^4	4.2	1.75×10^4	4.24	2.1×10^5	5.32
IGS	3.17×10^4	4.5	1.19×10^4	4.08	1.02×10^4	4.01	1.31×10^4	4.12
IGSy	3.66×10^3	3.56	2.69×10^3	3.43	2.92×10^3	3.47	7.67×10^3	3.88
IGrif vs. IG		4.3[a,b]		3.8[a,b]		3.76[a,b]		2.68[b]
IGS vs. IG		3.5[a,b]		3.92[a,b]		3.99[a,b]		3.88[a,b]
IGSy vs. IG		4.44[a,b]		4.57[a,b]		4.53[a,b]		4.12[a,b]
S. epidermidis ATCC								
IG	3.78×10^7	7.58	3.12×10^7	7.49	2.23×10^7	7.35	3.42×10^7	7.53
IGrif	7.73×10^3	3.89	1.42×10^4	4.15	1.64×10^4	4.21	5.54×10^4	4.74
IGS	3.8×10^3	3.58	9.9×10^2	3	1.66×10^4	4.22	3.01×10^3	3.48
IGSy	3.68×10^3	3.57	8.93×10^2	2.95	9.9×10^2	3	9.9×10^2	3
IGrif vs. IG		3.69[a,b]		3.34[a,b]		3.14[a,b]		2.79[b]
IGS vs. IG		4[a,b]		4.5[a,b]		3.13[a,b]		4.06[a,b]
IGSy vs. IG		4.01[a,b]		4.54[a,b]		4.35[a,b]		4.54[a,b]

Data are presented as colony forming units (CFU)/mL converted into log_{10}. The three antimicrobial grafts (IGrif, IGS, and IGSy) are compared with the one non-antimicrobial (IG) by using the log_{10} RF. ATCC = American type culture collection; IG = Intergard; IGrif = Intergard rifampicin soaked; IGS = Intergard Silver; IGSy = Intergard Synergy combining silver and triclosan; MRSA = methicillin-resistant Staphylococcus aureus; S. epidermidis = Staphylococcus epidermidis.

[a] Bactericidal efficacy at each exposure time as defined by approved guideline Clinical and Laboratory Institute Standards M-26A, 1999.

[b] Log_{10} reduction factor (RF).

Table 3. Growth of *E. coli*.

	24 h		48 h		72 h		168 h	
	Mean	Log_{10}/ Log_{10} RF	Mean	Log_{10}/ Log_{10} RF	Mean	Log_{10}/ Log_{10} RF	Mean	Log_{10}/ Log_{10} RF
E. coli ATCC								
IG	1×10^8	8	1×10^8	8	1×10^8	8	1×10^8	8
IGrif	7.96×10^7	7.9	9.53×10^7	7.98	1×10^8	8	1×10^8	8
IGS	2.87×10^3	3.46	2.65×10^3	3.42	1.41×10^3	3.15	1.4×10^3	3.15
IGSy	9.9×10^2	3	1.06×10^3	3.02	8.47×10^2	2.93	9.9×10^2	3
IGrif vs. IG		0.09[b]		0.02[b]		0[b]		0[b]
IGS vs. IG		4.54[a,b]		4.58[a,b]		4.85[a,b]		4.85[a,b]
IGSy vs. IG		5[a,b]		4.98[a,b]		5.07[a,b]		5[a,b]
E. coli clinical strain								
IG	1×10^8	8	1×10^8	8	1×10^8	8	1×10^8	8
IGrif	1.49×10^3	3.17	1.05×10^4	4.02	1.56×10^4	4.19	1.39×10^5	5.14
IGS	3.57×10^5	5.55	2.11×10^3	3.32	5.53×10^3	3.74	8.32×10^3	3.92
IGSy	5.2×10^4	4.72	8.31×10^2	2.92	2.36×10^3	3.37	2.08×10^3	3.32
IGrif vs. IG		4.83[a,b]		3.98[a,b]		3.81[a,b]		2.86[b]
IGS vs. IG		2.45[b]		4.68[a,b]		4.26[a,b]		4.08[a,b]
IGSy vs. IG		3.28[a,b]		5.08[a,b]		4.63[a,b]		4.68[a,b]

Data are presented as colony forming units (CFU)/mL converted into log_{10}. The three antimicrobial grafts (IGrif, IGS, and IGSy) are compared with the one non-antimicrobial (IG) by using the log_{10} RF. ATCC = American type culture collection; IG = Intergard; IGrif = Intergard rifampicin soaked; IGS = Intergard Silver; IGSy = Intergard Synergy combining silver and triclosan; E. coli = escherichia coli.

[a] Bactericidal efficacy at each exposure time as defined by approved guideline Clinical and Laboratory Institute Standards M-26A, 1999.

[b] Log_{10} reduction factor (RF).

Table 4. Growth of *C. albicans*.

	24 h		48 h		72 h		168 h	
	Mean	Log_{10}/ Log_{10} RF	Mean	Log_{10}/ Log_{10} RF	Mean	Log_{10}/ Log_{10} RF	Mean	Log_{10}/ Log_{10} RF
C. albicans ATCC								
IG	2.26×10^6	6.35	3.21×10^6	6.51	8.01×10^6	6.9	1×10^8	8
IGrif	1.85×10^6	6.27	4.18×10^6	6.62	9.8×10^6	6.99	1×10^8	8
IGS	8.75×10^3	3.94	1.32×10^4	4.12	3.04×10^4	4.48	1.45×10^4	4.16
IGSy	4.83×10^2	2.68	4.46×10^2	2.65	1.5×10^3	3.18	3.14×10^3	3.5
IGrif vs. IG		0.08[b]		−0.1[b]		−0.08[b]		0[b]
IGS vs. IG		2.41[b]		2.39[b]		2.42[b]		3.84[a,b]
IGSy vs. IG		3.67[a,b]		3.86[a,b]		3.73[a,b]		4.5[a,b]
C. albicans clinical strain								
IG	1.23×10^6	6.09	3.2×10^6	6.51	2.68×10^6	6.43	1×10^8	8
IGrif	8.01×10^5	5.9	1.8×10^6	6.26	3.89×10^6	6.59	1×10^8	8
IGS	4.18×10^3	3.62	1.15×10^4	4.06	2.69×10^4	4.43	2.25×10^4	4.35
IGSy	9.82×10^2	2.99	1.97×10^3	3.29	1.59×10^3	3.2	7.59×10^3	3.88
IGrif vs. IG		0.18[b]		0.25[b]		−0.16[b]		0[b]
IGS vs. IG		2.47[b]		2.44[b]		2[b]		3.65[a,b]
IGSy vs. IG		3.1[a,b]		3.21[a,b]		3.23[a,b]		4.12[a,b]

Data are presented as colony forming units (CFU)/mL converted into log_{10}. The three antimicrobial grafts (IGrif, IGS, and IGSy) are compared with the one non-antimicrobial (IG) by using the log_{10} RF. ATCC = American type culture collection; IG = Intergard; IGrif = Intergard rifampicin soaked; IGS = Intergard Silver; IGSy = Intergard Synergy combining silver and triclosan; C. albicans = candida albicans.

[a] Bactericidal efficacy at each exposure time as defined by approved guideline Clinical and Laboratory Institute Standards M-26A, 1999.

[b] Log_{10} reduction factor (RF).

those described in the clinical setting. Finally, looking at the study results, it can be concluded that exposing the patients to rifampicin soaked grafts, regardless of rifampicin concentration, appears not to be effective as the strains are at risk of emergence of resistance to the drug, as widely described in the literature. Subsequently, this strategy exposes patients to host rifampicin resistant mutants, which is a disastrous consequence since combination therapies using rifampicin are frequently prescribed to conservatively or post-operatively treat patients with infected vascular grafts. For instance, in a cohort of 84 patients with staphylococcal vascular graft infection, Legout et al.[26] have reported that antibiotic combination therapy containing rifampicin had better outcome than combinations without rifampicin (OR 0.32, 95% CI 0.10—0.96; $p = .04$). Although resistance to triclosan/silver or silver alone was not observed in the experiments, this could potentially arise as is the case for all selection pressures. The risk of collateral damage could not be excluded if resistance to triclosan and/or silver arise in a clinical strain as the genetic support of these resistances (i.e., plasmid) could host others resistance determinants. But unlike for rifampicin, this resistance would not compromise our antibiotic arsenal to the same extent.

Apart from rifampicin, some other antibiotic binding strategies have been studied. Jean-Baptiste et al.[27] have functionalised Dacron with cyclodextrin to allow a sustained drug eluting of ciprofloxacin vancomycin or rifampicin loaded separately. The bactericidal effect was further evaluated in vivo in a subcutaneous mouse model of local contamination of the implant by both Gram positive (*S. aureus*, *S. epidermidis*, MRSA) and Gram negative (*E. coli*, *Enterobacter cloacae*, and *Pseudomonas aeruginosa*) bacteria. Gram positive and Gram negative bacterial proliferations were significantly prevented by cyclodextrin dacron grafts loaded with rifampicin or with ciprofloxacin respectively. The authors concluded that they have demonstrated the efficacy of cyclodextrin dacron grafts loaded with appropriate antibiotics both in vitro and in vivo against six of the most common bacteria involved in human vascular graft infections. However, the difficulty preoperatively identifying the causative microorganism in order to select the appropriate antibiotic loaded graft, limits the routine implementation of these devices in clinical practice.

The study has the potential limitations of in vitro experiments. Even when the testing period was extended to seven days, the protocol remained a little far from the delicate post-operative course of infected vascular patients with the potential for multi-organ failure in the early period and potential for re-infection in the long term.[28] Large animal models could represent a valuable addition to these experiments but four vascular graft conditions separately inoculated by seven strains with at least six replicates in each group would be difficult to realise due to ethical and cost issues. Nevertheless, a polymicrobial infection scenario could be a great application for such in vivo models.

In conclusion, in the in vitro conditions, for all microorganisms tested, the Synergy graft combining silver with triclosan demonstrated a more sustainable and efficient seven day antimicrobial activity than the rifampicin soaked graft. Indeed, the combination of triclosan and silver enlarges the spectrum of activity (better coverage of fungi) and greatly reduces the risk of resistance development to either compounds (combination of the 2 compounds in

impregnated grafts, has a similar rationale as for drug combination therapy) even if resistance to triclosan was not observed in our experiments. The emergence of rifampicin resistant mutants suggests preference for a Synergy graft over a graft soaked in rifampicin to prevent or treat an infection when a biological solution is not feasible.

CONFLICTS OF INTEREST

X.B. received speaker fees from MAQUET-GETINGE to present preliminary results of this study during the Charing Cross meeting held in London in April 2017.

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APPENDIX A. SUPPLEMENTARY DATA

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ejvs.2018.08.053.

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