Efficacy of antiseptic impregnation of aortic endografts with rifampicin compared to silver against in vitro contamination with four bacteria that frequently cause vascular graft infections

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ABSTRACT

Objective: This in vitro study investigates the antimicrobial efficacy of impregnation of commercially available aortic endografts (EG) with rifampicin (RIF) and nanocolloidal silver.

Methods: Endografts were flushed with 50 mL of RIF 600 mg, 70 mL of a silver-based aqueous solution (AG), or 50 mL of phosphate-buffered saline (PBS) over 15 minutes. Endografts were then retrieved from the sheath and cut in 1 × 1 cm sized graft units (n = 80 of each impregnation), which were then incubated for 1 hour separately with inoculates containing 10^6 or 10^3 bacteria per milliliter (bact/mL) of each of the following bacteria: *Staphylococcus epidermidis, Escherichia coli,* multisensitive *Staphylococcus aureus*, and *Pseudomonas aeruginosa*. After sonication of the graft units, bacterial counts were measured by plating out twice the sonication solution on Mueller-Hinton plates.

Results: RIF showed a statistically significant decrease of colony forming units per milliliter for all four bacterial strains in both concentrations compared with PBS and AG, except for 10^3 bact/mL of *E coli*. AG showed a significant decrease of colony forming units per milliliter compared with PBS only for 10^6 bact/mL of *E coli* and was statistically significantly inferior to RIF for all four bacterial strains in both concentrations with the exception of *E coli* at a concentration of 10^3 bact/mL.

Conclusions: This in vitro study demonstrated infectivity resistance of aortic EG after flushing with RIF. Moreover, the feasibility of flushing aortic EG with a new silver-based agent could be demonstrated, but without statistically significant antimicrobial efficacy compared with native EG. (JVS—Vascular Science 2020;1:181-9.)

Clinical Relevance: Because the number of elective and emergency endovascular procedures on the thoracic and abdominal aorta is continuously increasing, endograft infections in both locations are also more frequently observed. The necessity and use of antimicrobial-impregnated EG in the prophylaxis or treatment of endograft infections has so far neither been discussed in the literature nor addressed in existing guidelines. This is the first study investigating the influence of antiseptic impregnations of commercially available aortic EG on in vitro contamination with bacteria commonly causing vascular graft infections. Owing to proven infectivity resistance, flushing EG with RIF could, despite known limitations, lead to a modification of established therapeutic principles in patients at high risk for aortic graft infections.

Keywords: Vascular graft infection; Endograft infection; Impregnation; Silver; Rifampicin

Endograft (EG) implantation in cases of potentially or confirmed infectious aortic pathologies has a high priority in emergency situations. In case of EG infection (EGI), patients and vascular surgeons are confronted with a complication that can be far more challenging and life threatening than the initial aortic disease. Early and aggressive therapy with complete removal of the infected EG is propagated as the best option.[1] However, owing to selection of patients for endovascular repair in the first place, the majority of patients are not suitable for EG explantation owing to poor general condition and relevant comorbidities.

The use of commercially available silver-impregnated grafts has become a frequent choice in open septic

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aortic surgery and the technique of soaking vascular grafts with rifampicin (RIF) has been used for decades for prophylaxis[2,3] and therapy[4-6] of prosthetic infections. However, there are currently no commercially available EG with antimicrobial impregnation.

Flushing an EG with RIF in cases of an expected increased risk of EGI (eg, systemic infection or bacteremia of nonaortic/vascular origin and nonpostponable aneurysm repair) or known infected aortic pathologies (eg, primary or secondary aortobronchial/aortoesophageal/aortoenteric fistula, mycotic aneurysm) is used in our department for bridging to open surgery or in palliative setting, although the antibacterial effectiveness of RIF impregnation has not yet been proven for EG. The known weak activity against gram-negative strains such as *Escherichia coli* and *Pseudomonas* species, methicillin-resistant *Staphylococcus aureus* (MRSA) and fungi,[5,7,8] as well as possible development of resistance[8,9] limits the use of RIF in most polymicrobially caused vascular graft infections.[6,10]

Silver as an antimicrobial agent has several advantages over RIF, including its wide antimicrobial activity and lack of resistance development.[9,11] The efficacy of silver acetate,[12-14] metallic silver,[15] and silver in combination with triclosan[9,16] has been proven in multiple clinical and experimental studies.

Therefore, with this in vitro trial, we tried to transfer silver as an established antimicrobial agent in open septic aortic surgery to endovascular procedures. The aim of this study was to confirm the antimicrobial efficacy of flushing EG with RIF and evaluate the feasibility and antimicrobial efficacy of flushing EG with nanocolloidal silver. Using the experimental protocol previously described elsewhere,[17] we compared both antimicrobial agents in an experimental model of simulated EGI.

ARTICLE HIGHLIGHTS

- **Type of Research:** This is an experimental research conducted in a single center with two different types of antiseptic agents as the independent variable and the bacterial colonization of commonly available aortic endoprostheses as the dependent variable
- **Key Findings:** There is infectivity resistance of endografts (EG) after flushing with rifampicin. Flushing EG with a new silver-based agent is feasible, but so far there is no evidence of statistically significant antimicrobial efficacy.
- **Take Home Message:** Irrigation of native EG with rifampicin may be useful in selected patients. However, owing to the development of bacterial resistance, other antiseptic impregnations need to be tested for their feasibility and antimicrobial properties in endovascular grafts, especially silver which antimicrobial efficacy in vascular grafts is well proven.

METHODS

Antibiotic agents and EG. Preparation of sterile RIF solution was as follows: 600 mg of RIF powder (Eremfat, Riemser Arzneimittel, Greifswald, Germany) was dissolved in 10 mL of PBS (270-300 mOsm/kg, pH 7.1-7.3, Gibco DPBS, Thermo Fisher Scientific, Waltham, Mass). After complete disappearance of the foam, the RIF solution was immediately added to a further 40 mL of PBS, so the final concentration was 12 mg/mL.

SILGEN Ag silver-based aqueous solution (AG; Genadyne Biotechnologies Hicksville, NY) is a sprayable solution of purified water and nanocolloidal silver with a silver content of greater than 30 ppm and was used undiluted. This CE (Conformité Européenne, French for European Conformity) approved class III medical device is licensed for use on chronic and surgical wounds.

Commercially available EG (Zenith Alpha Thoracic Endovascular Graft Distal Extension, Cook Medical Europe LTD, Limerick, Ireland) made of nitinol stents, lightweight woven polyester, and two types of suture material (green braided polyester and blue monofilament polypropylene), as well as gold radiopaque markers were used.

Bacterial strains. The bacterial isolates were 1585 wt for *S epidermidis*, ATCC29213 for MSSA, ATCC27853 for *P aeruginosa*, and ATCC25922 for *E coli*. Strains have been stored in a liquid medium at −80°C and were subcultured on tryptic Mueller Hinton agar according to the recommendations of the German Collection of Microorganisms and Cell Cultures GmbH (www.dsmz.de/). Two concentrations of bacterial suspensions (10^3 and 10^6 bact/mL) were prepared, determined using optical density at 600 nm ($OD_{600} = 0.2$ corresponds with 10^8 bact/mL).

Experimental protocol. This in vitro trial was conducted between April and December 2019 in the Institute for Medical Microbiology, Virology and Hygiene, University Medical Center Hamburg Eppendorf, Germany, following a predetermined study design. A schematic overview of the experimental protocol is portrayed in Fig 1.

In the first phase (impregnation), EG placed in the introduction system were flushed through the flush port with either, 50 mL of RIF 600 mg (12 mg/mL), 60 mL of AG, or a control of 50 mL of PBS (Gibco DPBS, Thermo Fisher Scientific) over 15 minutes. The impregnation time was adopted from protocols where standard grafts are soaked with RIF. EG were then retrieved from the sheath. In case of RIF, the orange color indicated an even distribution of the solution over the entire surface of the graft. No color was added to the silver solution for sterility reasons and to not affect its antimicrobial properties.

To facilitate further processing, nitinol stents were removed. Sutures and radiopaque markers were retained

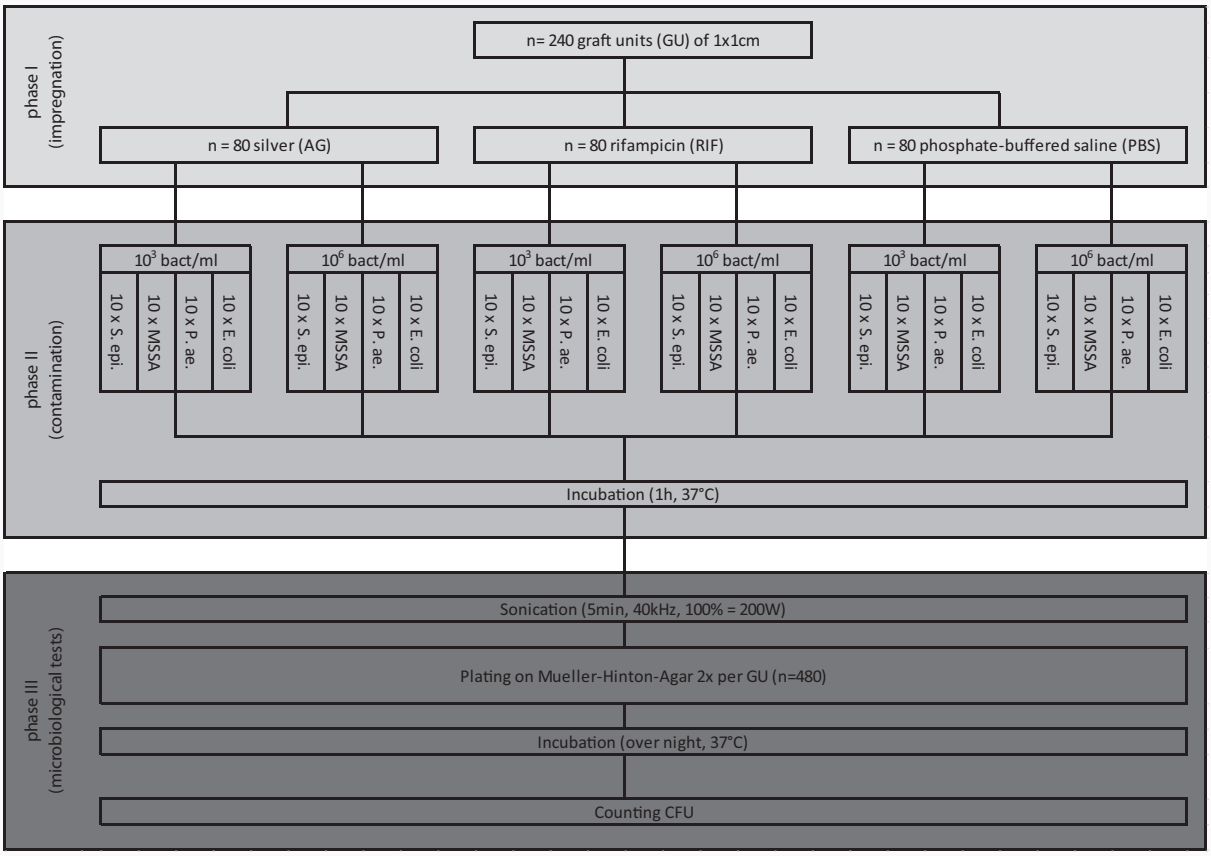

Fig 1. Experimental protocol. *bact/mL*, Bacteria per milliliter; *CFU*, colony forming units; *E coli*, *Escherichia coli*; *MSSA*, multisensitive *S aureus*; *P.ae.*, *Pseudomonas aeruginosa*; *S. epi.*, *Staphylococcus epidermidis*.

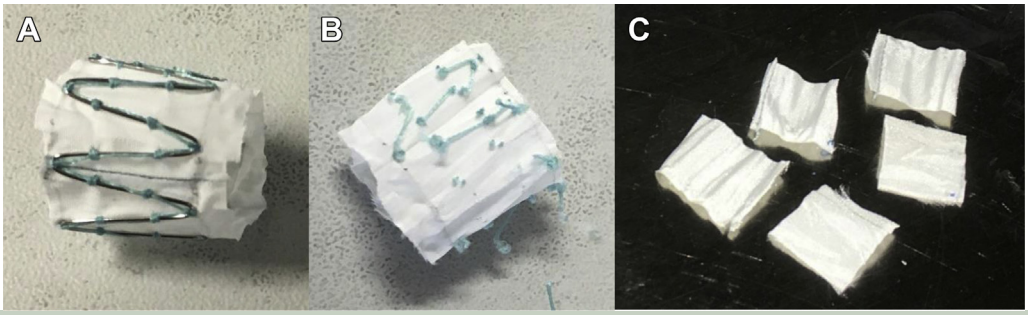

Fig 2. Cutting process. Part of the endograft (EG) after impregnation **(A)**, removal of the nitinol stents **(B)** and cutting into 1 × 1 cm pieces **(C)**.

on the fabric, which was then cut into 1 × 1 cm graft units (GU; Fig 2). Each piece was put into one 50-mL Falcon-tube (Fisher Scientific GmbH, Schwerte, Germany). All steps were performed under aseptic conditions in a microbiological safety workbench.

In the second phase (contamination), each Falcon tube was inoculated with 2 mL of a bacterial suspension in two concentrations (10^6 or 10^3 bact/mL) and the following four bacteria: MSSA, *S epidermidis*, *P aeruginosa*, and *E coli*. For each graft/microorganism combination, 10 repeated measures were carried out. This adds up to a total of 240 GU (10 GU × 3 impregnations × 4 bacterial strains × 2 inoculate concentrations). Falcon tubes were then incubated for 1 hour at 37°C. The untreated EG, flushed with PBS, served as positive controls.

During the third phase (microbiological tests), GU were retrieved from the bacterial solution and washed three times with 2 mL of clean PBS. Then sonication (Bacto-Sonic 14.2, Bandelin electronic GmbH & Co. KG, Berlin, Germany) of the GU was performed for 5 minutes at 40 kHz and 100% (200 W) for detachment and collection of any viable microorganisms potentially adhering to the

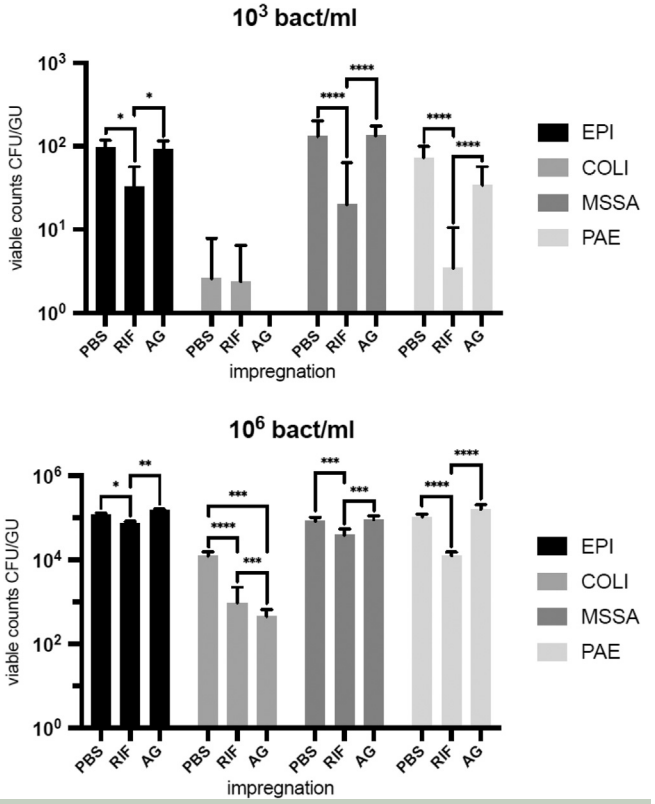

Fig 3. Average count of viable bacterial cells after contamination (1-hour incubation at 37°C) of impregnated GU with 10^3 and 10^6 bact/mL of *S epidermidis*, *E coli*, MSSA, and *P aeruginosa*, reflecting the antimicrobial efficacy of each agent. *AG*, Nanocolloidal silver solution; *CFU*, colony forming units; *COLI*, *Escherichia coli*; *EPI*, *Staphylococcus epidermidis*; *GU*, graft unit; *MSSA*, multisensible *Staphylococcus aureus*; *PAE*, *Pseudomonas aeruginosa*; *PBS*, phosphate-buffered saline; *RIF*, rifampicin. *$P < .05$; **$P < .01$; ***$P < .001$; ****$P < .0001$.

graft. GU were then taken out of the broth. One hundred microliters of the sonicated broth was either spread undiluted (10^3 bact/mL) or after serial 10-fold dilutions with saline (10^6 bact/mL) onto two fresh agar plates (Mueller-Hinton plates). All Mueller-Hinton plates were incubated over night at 37°C. The colony forming units (CFU) were then counted using the conventional plate count method.

Statistical analysis. Statistical analysis was performed and graphs were created with GraphPad Prism 8 (Graph Pad Software, LLC, San Diego, Calif). Quantitative culture results were presented as arithmetic mean ± 95% confidence interval. Blinding of the experiments was not possible owing to the orange color of the RIF. All assays were repeated 10 times, with the lowest and highest measures eliminated and the 8 remaining measures analyzed. The number of organisms were averaged as the mean CFU/mL. The averaged means were then converted and expressed as mean $\log_{10}$ CFU/mL. To determine a statistically significant difference between the viable mean $\log_{10}$ counts of the two graft impregnations, we used two-way analysis of variance with Bonferroni's multiple comparisons test. In accordance with the approved guideline CLSI M-26A, bactericidal activity was defined as a 3 log10 reduction of CFU/mL and bacteriostatic activity as a <3 log10 reduction of CFU/mL.[18] For all statistical tests, a *P* value of <.05 was considered statistically significant.

Ethical approval. No ethical approval was obtained because this study did not involve human participants, their tissue and/or data, or laboratory animals.

RESULTS

The viable mean $\log_{10}$ counts (CFU/mL; mean ± 95% confidence interval) of *S epidermidis*, *E coli*, MSSA, and *P aeruginosa* after contamination of GU with 10^3 and 10^6 bact/mL are illustrated in Fig 3.

Nonantimicrobial EG (PBS). The GU that were flushed solely with PBS showed no antimicrobial activity on any of the strains and served as controls.

EG impregnation with RIF. When contaminated with 10^3 bact/mL RIF showed bacteriostatic against all four bacterial strains with a statistically significant reduction of CFU/mL for *S epidermidis* ($P = .0338$), MSSA ($P < .0001$), and *P aeruginosa* ($P < .0001$) compared with PBS. For *E* coli, the decrease in CFU/mL was not significant compared with PBS (Table I).

When contaminated with 10^6 bact/mL, RIF also showed bacteriostatic activity and the reduction of CFU/mL was statistically significant for all four bacterial strains (*S epidermidis*, $P = .0447$; *E coli* $P < .0001$; *MSSA* $P = .0006$; and *P aeruginosa* $P < .0001$) compared with PBS (Table II).

RIF also caused a statistically significant reduction of CFU/mL for *S epidermidis* ($P = .0338$), *MSSA* ($P < .0001$), and *P aeruginosa* ($P < .0001$) in both bacterial concentrations compared with AG (Tables I and II). For *E* coli, a statistically significant reduction of CFU/mL compared with AG was only seen when the GU were contaminated with 10^6 bact/mL (Table II).

EG impregnation with nanocolloidal silver. When contaminated with 10^3 bact/mL, AG showed antimicrobial activity against *S epidermidis*, *P* aeruginosa, and *E coli*, but the decrease in CFU/mL was not statistically significant compared with PBS. However, it should be emphasized that zero CFU/mL could be detected when contaminated with 10^3 *E coli* ($P = .079$). For MSSA, CFU/mL were higher compared with PBS, but without statistical significance (Table I).

When contaminated with 10^6 bact/mL, AG showed antimicrobial activity against all four bacterial strains, but the reduction of CFU/mL was only significant for *E coli* ($P < .0001$) in terms of bacteriostatic activity (Table II). AG was statistically significant inferior to RIF for all four bacterial strains in both concentrations with

Table I. Two-way analysis of variance with Bonferroni's multiple comparisons test for 10^3 bacteria/mL

Bonferroni's multiple comparison test	Predicted LS mean difference	95% CI	Adjusted *P* value
Staphylococcus epidermidis			
PBS vs RIF	0.4725	0.02709-0.9179	**.0338**
PBS vs AG	0.01625	−0.4292 to 0.4617	>.9999
RIF vs AG	−0.4563	−0.9017 to −0.01084	**.0428**
Escherichia coli			
PBS vs RIF	0.0375	−0.4079 to 0.4829	>.9999
PBS vs AG	0.4125	−0.03291 to 0.8579	.0787
RIF vs AG	0.375	−0.07041 to 0.8204	.1284
MSSA			
PBS vs RIF	0.8175	0.3721-1.263	**<.0001**
PBS vs AG	−0.0075	−0.4529 to 0.4379	>.9999
RIF vs AG	−0.825	−1.270 to −0.3796	**<.0001**
Pseudomonas aeruginosa			
PBS vs RIF	1.326	0.8843-1.768	**<.0001**
PBS vs AG	0.3313	−0.1107 to 0.7732	.2119
RIF vs AG	−0.995	−1.437 to −0.5531	**<.0001**

AG, Nanocolloidal silver solution; *CI*, confidence interval; *LS*, least squares; *MSSA*, multisensitive *S aureus*; *PBS*, phosphate-buffered saline; *RIF*, rifampicin.
Boldface entries indicate statistical significance.

the exception of *E coli* at a concentration of 10^3 bact/mL, where neither of the two impregnations showed a significant reduction in CFU/mL compared with PBS. However, because zero viable counts were detected under AG, there is a trend toward the superiority of silver compared with RIF at 10^3 *E coli* (Tables I and II).

DISCUSSION

EG impregnation with RIF. RIF soaking has established itself in open septic aortic surgery owing to numerous proofs of its effectiveness,[4-6] but the microbial effect when used in EG has not been tested yet. Because gram-positive bacteria are the most common pathogenic of both primary aortic and secondary graft infections,[4] our practice has its justification despite the well-known limitations against gram-negative germs, MRSA, and fungi.[5,7,8]

With a significant decrease of CFU/mL for 10^3 bact/mL of *S epidermidis*, MSSA, and *P aeruginosa* and for 10^6 bact/mL of *S epidermidis*, MSSA, *P aeruginosa*, and *E coli* compared with PBS we were able to prove the antimicrobial effectiveness of this technique in EG experimentally for the first time. The lack of a significant reduction of CFU/mL at an inoculation of grafts units with 10^3 bact/mL *E coli* compared with 10^6 bact/mL *E coli* is most likely owing to the fact that the number of viable counts was significantly decreased in all impregnations, even PBS, compared with the other bacteria at this concentration (Fig 3).

Both the concentration (12 mg/mL) and the flushing time (15 minutes) used in our experimental setup were based on numerous publications with concentrations between 1 and 60 mg and 15 and 30 minutes. All studies had in common that gelatin-coated polyester grafts were used,[4-7,9,16,19,20] because gelatin has been shown to offer the best antibiotic binding properties.[19] Commercially available EG are either made of polyester, as used in this study, or of polytetrafluoroethylene without gelatin pretreatment. To reflect reality, no changes have been made to the fabric of the EG used, and to our knowledge, no data are available on the recommended dose and flushing time of RIF for both pure polyester grafts and EG.

The lack of gelatin impregnation might explain why we only detected bacteriostatic activity, although bactericidal activity of RIF-soaked gelatin-treated polyester grafts had previously been demonstrated.[9] Therefore, it would theoretically be interesting to repeat the experiment with an EG generation made of gelatin-treated polyester to support the clinical use of RIF treatments of EG. However, we are not aware that such a product is available or planned by any manufacturer. Possible reasons for this could be changes in packing, sterilization, and the durability of EG. In addition, sealing of the pores, which is essential in open surgery, is not necessary for EG, since intraoperative type IV endoleaks usually disappear after normalization of coagulation.

Furthermore, because polyester has been found to bind antibiotics more effectively than polytetrafluoroethylene,[19] we suggest that, although the flushing process

Table II. Two-way analysis of variance with Bonferroni's multiple comparisons test for 10^6 bacteria/mL

Bonferroni's multiple comparison test	Predicted LS mean difference	95% CI	Adjusted *P* value
Staphylococcus epidermidis			
PBS vs RIF	0.2087	0.003603-0.4139	**.0447**
PBS vs AG	−0.1038	−0.3089 to 0.1014	.6663
RIF vs AG	−0.3125	−0.5176 to −0.1074	**.0011**
Escherichia coli			
PBS vs RIF	1.118	0.9124-1.323	**<.0001**
PBS vs AG	1.439	1.234-1.644	**<.0001**
RIF vs AG	0.3213	0.1161-0.5264	**.0008**
MSSA			
PBS vs RIF	0.3263	0.1211-0.5314	**.0006**
PBS vs AG	−0.03625	−0.2414 to 0.1689	>.9999
RIF vs AG	−0.3625	−0.5676 to −0.1574	**<.0001**
Pseudomonas aeruginosa			
PBS vs RIF	0.9238	0.7186-1.129	**<.0001**
PBS vs AG	−0.1813	−0.3864 to 0.02390	.1013
RIF vs AG	−1.105	−1.310 to −0.8999	**<.0001**

AG, Nanocolloidal silver solution; *CI*, confidence interval; *LS*, least squares; *MSSA*, multisensitive *S aureus*; *PBS*, phosphate-buffered saline;*RIF*, rifampicin.
Boldface entries indicate statistical significance.

can in principle be performed with other brands of EG, the results are only transferable to EG where the fabric is also made of polyester.

EG impregnation with nanocolloidal silver. The idea of flushing EG with a nanocolloidal silver solution arose from the well-known limitations of RIF and this is the first experimental trial, in which standard EG are impregnated with this new antiseptic agent. Also, the flushing process could be achieved identically with different brands of EG. However, there are two decisive points that could explain the lack of a statistically significant effect of nanocolloidal silver in this in vitro model. First, a reduced binding of silver to the graft: The sprayable silver solution, approved for use on external and internal wounds, was used for irrigation of the stent grafts. The purified water contained in the silver solution allows it to reach every unevenness and different depth of the wound bed when applied as a spray. It evaporates within 2 minutes after application. The use as a solution and not as a spray may, therefore, not lead to the same effect, because the silver does not adhere to the surface in the same way during the flushing process as during spraying. In addition, the adherent silver particles may be detached manually during the cutting process. A modification of the flushing process with longer flushing and larger volume may change this. Doubling the flushing time to 30 minutes is at the limit of what is feasible in a comparable situation in hybrid operating room, especially because infected aortas are usually urgent or emergency cases. For the same reason, sterile deployment of EG on a side table, spraying on of the silver solution and time-consuming resheathing are not recommended. Also the even distribution of the silver solution over the entire EG during the rinsing process cannot be proven owing to the lack of color. However, because it was also a water-based solution, an equal distribution was assumed, similar to that of the RIF solution.

Second, the exposure time of silver may have been to short. The antimicrobial mode of action of silver ions is complex and not yet fully understood. Silver ions impair bacterial cell wall integrity, bind and disrupt subcellular components, and inactivate bacterial DNA and RNA and, therefore, damage essential protein synthesis and metabolic events.[21,22] The efficacy of silver ions is also proven to be higher against gram-negative bacteria like *E coli* then against gram-positive *S aureus*, possibly owing to higher thickness of the peptidoglycan layer, which may prevent the action of the silver ions through the bacterial wall.[22] This factor may have caused the ineffectiveness of AG at 10^3 bact/mL of *MSSA* compared with PBS with even higher CFU/mL for AG. Furthermore, it is conceivable that, especially for the molecular biological processes, the incubation time of the grafts with each bacterial suspension of 1 hour might have been too short, which may explain that statistically significant reduction of viable counts could only be demonstrated for 10^6 bact/mL of *E coli*. However, in preliminary experiments, longer incubation periods of up to 24 hours with 10^6 bact/mL and native EG (flushed with PBS) were also investigated, but the longer the incubation time, the more likely bacteria died owing to apoptosis without antimicrobial

agents. Here, *P aeruginosa* as an environmental germ showed the lowest sensitivity to longer incubation, whereas *S epidermidis* showed the highest reduction of CFU. For this reason and for reasons of practicability, an incubation time of 1 hour was determined.

Furthermore, for 10^3 of *E coli*, no significant decrease of CFU/mL could be shown after silver impregnation, as with RIF, although no viable counts could be detected at all. This seems to be caused by the strongly reduced number of viable counts in all impregnations compared with the other bacteria (Fig 3).

Evaluation of the described practice with regard to prophylaxis of EGI. An increased risk of an EGI is difficult to assess because there is no common risk score. In cases of bacteremia of nonaortic/vascular origin, an increased risk of EGI can generally be assumed and EG implantation should be delayed. However, sometimes aortic treatment cannot be postponed, despite systemic infection. The incubation time of 1 hour with 10^3 or 10^6 bact/mL in this trial seems acceptable for the evaluation of antiseptic impregnation in regard to prophylaxis for EGI. Routine flushing of EG with RIF for prophylaxis of EGI should be rejected owing to the development of bacterial resistance, but is in our opinion justifiable in selected cases given the lack of commercially available antiseptic impregnated EG. Owing to the proven efficacy of silver against *E coli* (zero CFU/mL) when contaminated with 10^3 *E coli* ($P = .079$) and statistically significant reduction of CFU/mL when contaminated with 10^6 *E coli* ($P < .0001$), a combination of RIF and silver seems to be useful in suspected or verified systemic *E coli* infection.

Evaluation of the described practice with regard to treatment of primary or secondary infected aortic pathologies. Although open surgical repair is supported as the gold standard for mycotic aortic aneurysms in current aortic aneurysm guidelines, endovascular repair is recommended as an alternative[23,24] owing to early survival benefit up to 4 years postoperatively.[25] We consider EG impregnation with RIF in such cases as legitimate in addition to systemic antibiotic therapy.

The incidence of EGI after EVAR is <1%.[26,27] Although the joint incidence for thoracic graft infections after open and endovascular aortic repair is reported to be up to 6%,[28] the incidence of thoracic EGI is so far unknown. New endoleaks and aortobronchial/pulmonary or aortoenteric fistulas are feared complications of an EGI with incidences of 0.56%[29] and 0.3% to 2.0%,[30] respectively. Emergency EG insertion as a primary lifesaving procedure is proposed to control exsanguination and restore hemodynamic stability.[31] But leaving the primary infected material can cause septic complications and recurrent bleeding, so this strategy is mainly seen as a bridging to open surgical repair.[28] Given the invasiveness of such procedures with mortality rates of 21% to 56% at 1 year for abdominal EG removal[32,33] and 73% and 71% at 2 and 5 years, respectively, after thoracic EG removal,[10,34,35] a significant proportion of patients with thoracic and abdominal EGI are not suitable candidates for open surgery. Implantation of antimicrobial impregnated EG as a palliative concept could be an option to improve outcome in this fragile patient cohort. However, we admit that the incubation period of one hour in this study seems to be insufficient to evaluate the bactericidal activity of EG in case of implantation in an infected field.

Limitations. The main limitation of our results is the possibility of short-term or mid-term microbial selection or resistance when using RIF, which we did not notice because the antiseptic efficacy of RIF was not investigated over time. So, recently published data by Berard et al[9] on in vitro testing of differently contaminated RIF soaked polyester graft showed a gradually decrease in the primary bactericidal effect of RIF within 7 days owing to the development of resistance, not only with MRSA and *E coli*, but also with typical RIF-sensitive strains like *S epidermidis*.[9]

In addition, removing the nitinol stents before cutting in our experimental protocol was done for reasons of practicability and cost efficiency, so we could also use the parts of the EG that were without nitinol stents. We justified this practice with the rarity of reported bare metal stent infections of about 100 cases since 1966, which are numerically disproportionate to reported infected covered metal stents.[36,37] However, the in vitro behavior of GU does not automatically reflect antimicrobial resistance or susceptibility of the entire EG.

Finally, these limitations exist with regard to evaluation of a therapeutic approach to primary or secondary aortic infections.

CONCLUSIONS

This in vitro study for the first time confirms the antimicrobial efficacy of flushing aortic EG with RIF in term of infectability resistance. Given the lack of available antiseptic impregnated EG, flushing native EG with RIF might be useful in selected patients, even if a benefit in vivo can only be proven by clinical studies. However, because the development of bacterial resistance to RIF has been demonstrated in the past and multiresistant germs are one of the main problems of current public health, other antiseptic impregnations need to be evaluated for their feasibility and antimicrobial properties in endovascular grafts. Because, in this model, flushing EG with a new silver-based agent did not show promising results, although the antimicrobial efficacy of silver is well proven in vascular grafts, further studies on EG impregnation with silver should be requested.

Genadyne Biotechnologies provided the *SILGEN Ag* silver spray, but had no role in data collection, data analysis, data interpretation or writing of the report. The EG used

were purchased for patient care purposes within the normal hospital budget, but had not been used on patients and were therefore still in their original packaging. The authors are grateful to Anna Both for her great help in conducting the microbiological tests and to Gerhard Schoen for his help in statistical analysis.

AUTHOR CONTRIBUTIONS

Conception and design: SH, PS, HR, HD
Analysis and interpretation: SH, PS, HR, TK, ESD, HD
Data collection: SH, PS, HR, HD
Writing the article: SH
Critical revision of the article: SH, PS, HR, TK, ESD, HD
Final approval of the article: SH, PS, HR, TK, ESD, HD
Statistical analysis: SH, PS
Obtained funding: Not applicable
Overall responsibility: SH

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