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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 $\log_{10}$ reduction factor (logRF). To calculate the overall difference in the mean $\log_{10}$ 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 reconstructions[1] 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 veins[4] or cryopreserved arterial homografts[5] seems a logical option if the aim is to prevent recurrent infection.[6] However, cryopreserved arterial homografts are available in limited quantities[7] 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 results[20] 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