Revised 13/10/2021

Stapled porcine pericardium displays lower infectivity *in vitro* than native and sutured porcine pericardium

Benjamin Del Tatto, MD[1,3,4], Didier Le Roy, PhD[2], Martine Lambelet, MD[1], Jean-Marc Corpataux MD, PhD[1], Nabil Chakfé, MD, PhD[3,4], Stefano Giulieri, MD, PhD[2], Florent Allagnat, PhD[1*], Thierry Roger, PhD[2*] and François Saucy, MD, PhD[1*]

[1]Department of Vascular Surgery, Lausanne University Hospital and University of Lausanne, Lausanne, Switzerland

[2]Infectious Diseases Service, Department of Medicine, Lausanne University Hospital and University of Lausanne, Epalinges, Switzerland

[3]Department of Vascular Surgery and Renal Transplantation, University of Strasbourg, Strasbourg, France

[4]Groupe Européen de Recherche sur les Prothèses Appliquées à la Chirurgie Vasculaire (GEPROVAS), CHRU, Strasbourg, France

*These authors contributed equally to this work (co-senior authorship).

Corresponding author: François Saucy, BH-10.159, Centre Hospitalier Universitaire Vaudois, Rue du Bugnon 46, CH-1011 Lausanne, Switzerland. François.Saucy@chuv.ch; Tel: + 41 21 314 02 35

Running title: Low infectiveness of stapled porcine pericardium

Word count: 2610 words

Category: Original article

AUTHOR CONTRIBUTIONS STATEMENT

BDT, DLR, SG, FA, TR and FS designed the study. BDT, DLR and ML performed the experiments. BDT, DLR, FA and TR analyzed the data. FA, TR and FS wrote the manuscript. JMC and NC critically revised the manuscript. FA, TR and FS finalized the manuscript. All the authors revised the manuscript.

28

Abstract (247 words)

Background: Biological xenografts using tubulized porcine pericardium are an alternative to replace infected prosthetic graft. We recently reported an innovative technique using a stapled porcine pericardial bioconduit for immediate vascular reconstruction in emergency. The objective of this study was to compare the growth and adherence to grafts of bacteria and yeast incubated with stapled porcine pericardium, sutured or naked pericardium.

Materials & Methods: One square centimeter of porcine pericardial patches, with or without staples or sutures, was incubated with 10^5 colony forming units (CFU) of *Escherichia coli*, *Staphylococcus aureus*, *Staphylococcus epidermidis* and *Candida albicans* for 1, 6 and 24 hours. The medium was collected to quantify planktonic microorganisms, while grafts were sonicated to quantify adherent microorganisms. Dacron and Dacron silver were analyzed in parallel as synthetic reference prostheses.

Results: Stapled porcine pericardium reduced the growth and the adherence of *E. coli* (2 to 30-fold; $p < .0005$), *S. aureus* (11 to 1000-fold; $p < .0006$), *S. epidermidis* (> 500-fold; $p < .0001$) and *C. albicans* (12 to 50-fold; $p < .0001$) when compared to medium alone (growth) and pericardium or Dacron (adherence). Native and sutured porcine pericardium interfered with the growth and the adherence of *E. coli* and *C. albicans*, and Dacron with that of *S. epidermidis*. As expected, Dacron silver was robustly bactericidal.

Conclusions: Stapled porcine pericardium exhibited a lower susceptibility to infection by bacteria and yeasts *in vitro* when compared to the native and sutured porcine pericardium. Stapled porcine pericardium might be a good option for rapid vascular grafting without increasing infectivity.

Keywords (3-6): Pericardium, Vascular graft, Infection, Adherence, Dacron

Highlights

- Porcine pericardial xenograft shows reduced bacterial growth and adherence compared to Dacron grafts.
- Dacron Silver graft is strongly bactericidal, but only mildly fungistatic
- Porcine pericardial xenograft with staples interferes with the growth and the adherence of bacteria and yeast better than naked or sutured-stapled pericardium.
- Tubulized stapled porcine pericardium might represent an alternative to synthetic vascular grafts for rapid vascular replacement and possibly for patients with a high risk of infection.

INTRODUCTION

Infection of vascular graft is a rare, severe complication of open surgical revascularization, associated with patients' comorbidities, surgical and environmental factors. The incidence of vascular graft infection ranges from 2% for aortic grafts to 4% for femoropopliteal grafts, while the mortality rate oscillates from 24% to 75%.[1,2] The main microorganisms responsible for infections are Gram-positive bacteria, including *Staphylococcus aureus*, coagulase negative staphylococci and enterococci, and Gram-negative bacteria. Fungal infections are common, especially in aortic location, and are associated with severe complications such as mycotic aneurysms, graft reinfection and graft rupture.[1, 2]

The management of infected vascular grafts is still debated. Systemic antibiotherapy usually comes with partial or total graft explantation, followed by *in situ* reconstructions.[1, 2] Biological allografting performed with cryopreserved veins or arteries and autologous deep femoral veins is the preferred option to prevent reinfection. However, such grafts may not be available for emergency intervention. In rare cases, they also suffer from early mechanical failure due to rupture or thrombosis.[3-7] Synthetic graft materials such as non impregnated polyester prostheses (Dacron) are not recommended as they present a high-risk of recurrent infection. Finally, the use of antimicrobial vascular grafts have shown mixed results due to reinfection by emerging antimicrobial-resistant miroorganisms.[8-11]

Pericardial patches of both bovine and porcine origin have been used in cardiovascular surgery over the last two decades. Pericardial patches are resistant and available in handy sizes. Vascular reconstruction using pericardial tubes wrapped with continuous suture show a low rate of reinfection when compared to the synthetic vascular prosthesis, constituting a promising alternative to synthetic graft .[12-18] To avoid this limitation, we recently reported a stapler-made bioconduit using a porcine pericardial patch, prepared in a few minutes, used for *in situ* vascular reconstruction after infected graft removal.[19] The possible benefits of using porcine pericardium tubes over synthetic grafts are mainly

deduced from clinical observations and the assumption that biological grafts have an increased resistance toward infection.

Yet, a limited number of studies assessed the growth and adherence of microorganisms to vascular graft materials, and none evaluated the susceptibility of the stapled porcine pericardium to bacterial infection. Our study aimed to compare *in vitro* bacterial and fungal growth and adherence to stapled porcine pericardium with native and sutured pericardium. Polyester (Dacron) and polyester silver (Dacron Silver) were used as reference material for their well-characterized response to bacterial and fungal growth and adherence *in vitro*.

MATERIALS AND METHODS

Microorganisms

Escherichia coli O18:K1:H7,[20] methicillin-resistant *Staphylococcus aureus* Rosenbach (American Type Culture Collection, Manassas, VA, USA; ATCC 33591) and *Staphylococcus epidermidis* Evans (ATCC 12228) were cultured at 37 °C in Mueller Hinton broth (BD, Franklin Lakes, NJ, USA). *Candida albicans* Berkhout (ATCC 90028) was cultured at 30 °C in Sabouraud broth (BD). The growth of bacteria and *C. albicans* was recorded by measuring OD 620 nm and OD 540 nm using a nephelometer (NovaSpec™ Plus, Amersham Biosciences). Microorganisms were collected during exponential growth, washed in phosphate-buffered saline, and adjusted to 10^5 colony forming units (CFU)/mL Mueller Hinton broth or Sabouraud broth.

Graft material

Graft material consisted of 1) porcine pericardium (No-React®Patch, BioIntegralSurgical, Mississauga, Canada), 2) porcine pericardium with staples (Endopath Echelon™ 60 mm Reloads Thin GST60W, Ethicon®Inc, Somerville, MA, USA) applied using an Echelon Flex (Ethicon®Inc, Somerville, MA, USA), 3) porcine pericardium with sutures (2 loops of 5 knots by 5.0 polypropylene), 4) polyester (Intergard®, Maquet, Getinge Group, Rastatt, Germany) and, 5) polyester silver (Intergard Silver®, Maquet).

Growth and adherence to vascular grafts of microorganisms

One cm^2 of vascular grafts or six staples were incubated for 1, 6 and 24 h at 30 or 37 °C under shaking (100 revolutions per minute) in 1 mL Mueller Hinton broth or Sabouraud broth containing 10^5 CFU bacteria or *C. albicans*. Serial dilutions of supernatant were plated on Mueller Hinton blood agar plates (BD) to quantify the planktonic growth of microorganisms. Grafts were washed three times in 2 mL 0.9% sodium chloride, transferred to a tube containing 1 mL Mueller Hinton or Sabouraud broth, and sonicated 5 min using an ultrasonic water bath (TPC120, 30KHz,

126 TELSONIC Ultrasonics, Bronschhofen, Switzerland) for maximal recovery of adhering
127 microorganisms.[21] Serial dilutions of medium were plated on Mueller Hinton blood agar
128 plates (BD). Colonies were numerated after 24 h of incubation at 30 or 37 °C. We
129 compared the infectivity and antimicrobial activity of three biological vascular
130 xenografts, porcine patches with or without staples or sutures (**Fig. 1**). Polyester
131 (Dacron) and polyester silver (Dacron Silver), which displays strong bactericidal
132 activity, were used as reference synthetic vascular prosthesis material.

133 **Statistical analysis**

134 Procedures were repeated eight times for each graft and microorganism, except for polyester
135 silver that was tested 3-5 times as we expected a strong effect of the graft on bacterial growth.
136 The experimental design was based on published studies aimed at reaching power of 80%.[11,
137 22] Data were analyzed using PRISM version 8.4.0 software (GraphPad Software, La Jolla,
138 CA, USA). Data were log-transformed to normalize the distribution.[23] Comparisons between
139 groups were made using ordinary one-way ANOVA test, followed by multiple comparisons
140 using post-hoc t-test with Tukey's correction for multiple comparisons. *p* values of less than
141 .05 were considered to indicate statistical significance. *$p \leq .05$; **$p \leq .01$; ***$p \leq .001$; ****$p \leq$
142 .0001.

RESULTS

Reduced growth and adherence of *E. coli* to pericardium grafts

The CFU of planktonic *E. coli* increased 7.4 x 10^3-fold after 6 h of incubation in medium (**Fig. 2, Table S1**). The presence of stapled pericardium, pericardium and sutured pericardium hindered the growth of *E. coli* by 30% to 50% when compared to medium and Dacron that, as expected, did not affect the growth of *E. coli* ($p < .0005$ for all conditions). A small fraction of *E. coli*, 0.01-0.2% of total counts, adhered to the grafts (**Fig. 2, Table S2**). The numbers of *E. coli* adhering to the pericardium, stapled pericardium and sutured pericardium were 17 to 30-fold lower than for Dacron ($p < .0001$). As anticipated, Dacron Silver was bactericidal and reduced 10^4-10^7-fold the number of planktonic and adherent *E. coli* ($p < .0001$). Similar results were obtained after 1 h of incubation, while all but with polyester silver cultures of *E. coli* were saturated after 24 h of incubation.

Reduced growth and adherence of *S. aureus* to stapled pericardium grafts

The CFU of *S. aureus* increased 175-fold and 2.6 x 10^3-fold after 6 h and 24 h incubation in medium, respectively (**Fig. 3, Table S3**). Stapled pericardium reduced 60 and 7-fold the growth of *S. aureus* after 6 h and 24 h of incubation, respectively ($p = .0002$ and $p = .0006$), while pericardium, sutured pericardium and Dacron had no impact on bacterial growth ($p > .05$). After 24 h, growth inhibition by stapled pericardium was substantial when compared to pericardium (11-fold reduction, $p = .0002$), sutured pericardium (10-fold, $p = .0007$) and Dacron (12-fold, $p = .0002$). Around 0.2-1% of *S. aureus* adhered to the grafts (**Fig. 3**). After 6 h and 24 h of incubation, the numbers of *S. aureus* adhering to stapled pericardium was 1000-fold lower than for Dacron ($p < .0001$ at both 6 h and 24 h, **Table S4)**. The decrease was less marked for pericardium (20-35-fold, $p = .018$ and $p = .16$) and not statistically significant for sutured pericardium (3.5-22-fold, $p = .18$ and $p = .38$, **Table S4**). Dacron Silver was

bactericidal and decreased 1-5 x 10^4-fold the number of planktonic and adherent *S. aureus* recovered after 6 h. Less than 10 CFU of *S. aureus* were measured after 24 h (**Fig. 3**).

Reduced growth and adherence of *S. epidermidis* to stapled pericardium grafts

The CFU of *S. epidermidis* increased 10-fold and 3.7 x 10^3-fold after 6 h and 24 h incubation in medium (**Fig. 4**). Apart from the naked pericardium, all grafts tended to decrease the growth of *S. epidermidis* after 6 h, but only stapled pericardium had a statically significant effect (550-fold reduction, $p < .0001$, **Table S5**). The stapled pericardium effect was significant after 24 h of incubation ($p < .0001$), while pericardium and sutured pericardium did not affect bacterial growth. Surprisingly, Dacron strongly reduced *S. epidermidis* growth after 24 h ($p < .0001$ *vs* medium, $p = .0012$ *vs* stapled pericardium, **Table S5**).

Approximately 0.2-0.5% of *S. epidermidis* adhered to the pericardium (**Fig. 4**). After 6 h of incubation, no bacteria were detected onto stapled pericardium and Dacron Silver. Twenty-fold more *S. epidermidis* adhered to pericardium and 2-fold more to sutured pericardium than to Dacron ($p = .15$ and $p = .96$, **Table S6**). After 24 h of incubation, 500 and 10^5-fold more *S. epidermidis* adhered to pericardium or sutured pericardium than to stapled pericardium ($p = .0002$ and $p < .0001$) and Dacron ($p < .005$ and $p < .0001$). Because planktonic growth was strongly reduced by stapled pericardium and Dacron, the number of *S. epidermidis* adhering to the grafts represented as much as 2 to 5% of all living bacteria. Dacron Silver was bactericidal and fully abrogated bacteria adherence after 6 h and 24 h of incubation (**Fig. 4, Table S6**). The data obtained from cultures of *S. aureus* and *S. epidermidis* suggested a possible bactericidal effect of the stapled pericardium (**Fig 3** and **4**). Therefore, we tested whether staples interfered with bacterial growth. Staples alone had no impact on the recovery of *S. aureus* and *S. epidermidis* after 6 h and 24 h of incubation (**Fig. 5**).

Reduced growth and adherence of *C. albicans* to stapled pericardium grafts

195 The CFU of *C. albicans* increased 17 and 270-fold after 6 h and 24 h incubation in medium
196 (**Fig. 6**). Dacron did not hinder the growth of *C. albicans* after 6 h and 24 h of incubation when
197 compared to medium alone. Pericardium, stapled pericardium and sutured pericardium
198 reduced the growth of *C. albicans* 20 to 50-fold after 6 h ($p \leq .0006$ *vs* medium or *vs* polyester,
199 **Table S7**). After 24 h, pericardium tended to reduce the growth of *C. albicans* ($p = .061$; **Fig.**
200 **6**). Of note, Dacron Silver was fungistatic and resulted in the lowest growth rate of *C. albicans*
201 growth after 6 h ($p < .0001$) but not after 24 h of incubation (**Table S7)**. As much as 5% of *C.*
202 *albicans* adhered to Dacron (**Fig. 6**). In contrast, only 0.2-0.5% of *C. albicans* adhered to
203 pericardium, stapled pericardium, sutured pericardium and Dacron Silver. Compared to
204 Dacron, all pericardial grafts reduced yeast adherence after 6 h (3 to 7 fold reduction, **Table**
205 **S8**) and 24 h (12 to 50 fold reduction, **Table S8**) to levels comparable to that of Dacron Silver.
206

DISCUSSION

The present study suggests that, *in vitro*, stapled porcine pericardium decreases the growth and adherence of bacteria and yeast compared to native or sutured porcine pericardium. This observation is interesting considering that the usage of stapled pericardial tube is a quick and easy alternative procedure for vascular reconstruction in acute conditions during which biological substitutes are not immediately available.[19]

A limited number of studies assessed the growth and adherence of microorganisms to vascular graft materials and none to the porcine pericardium. Here, we report that the porcine pericardium, despite no antibacterial properties, lowered *E. coli* and *S. aureus* growth. That said, the porcine pericardium still showed high growth and adherence compared to the antimicrobial Dacron Silver graft, which consistently displayed potent bactericidal properties against all strains. Surprisingly, all types of pericardium patches showed significantly reduced adherence of *C. albicans* compared to the native Dacron graft, down to levels similar to those obtained with Silver graft. However, it should be noted that Dacron Silver had no potent antifungal activity, and reduced by only 30% *C. albicans* adherence compared to the Dacron. Our results support previous studies showing that silver does not reduce *C. albicans* growth on vascular prostheses, and may even promote the formation of biofilm.[24, 25] Overall, the pericardium grafts perform better than the Dacron graft in terms of bacterial growth and adherence, except for *S. epidermidis*. The pericardium grafts perform as well as the Silver graft for *C. albicans*.

A legitimate concern when using staples is that it might increase the risk of infection compared to traditional stitches. Here, we consistently observed that stapled porcine pericardium showed lower growth and the adherence of *E. coli*, *S. aureus*, *S. epidermidis* and *C. albicans* than native and sutured porcine pericardium. The presence of staples somehow reduced the growth and attachment of *S. aureus* and *S. epidermidis*, although staples alone had no bacteriostatic effect on staphylococci. Although further studies are required to elucidate

the reason behind this effect, these findings support the safety of stapled porcine pericardium tubes as a quick alternative to sutured pericardial tubes.

Inhibition of microbial growth after 6 h of incubation with stapled porcine pericardium was lower for *E. coli* than for *S. aureus*, *S. epidemidis* and *C. albicans*. Concurrently, after 6 h incubation in medium alone, *E. coli* multiplied 40, 740 and 450-fold more than *S. aureus*, *S. epidemidis* and *C. albicans*, respectively. Thus, the reduced impact of the stapled porcine pericardium on *E. coli* was likely related to the fast-growing performance of the bacteria. Supporting this hypothesis, all the 24 h growth assays were saturated with *E. coli*, except when using powerfully bactericidal polyester silver.

Our study has several limitations. We have tested a limited number of pathogens in an *in vitro* assay. It will be interesting to increase our panel of bacteria and fungi, including especially strains recovered from recurrent infections. In this study, we used flat patches of material, without wrapping to avoid construction and geometry biases. We should also enlarge the panel of graft material to be compared and even assess the effects of the combination of different materials. Indeed, the creation of long bifurcated stapled tubulized grafts will require potentially circular polypropylene sutures to join tubes together. Finally, animal models could be used to confirm our observations in settings that more closely mimic the complex pathophysiological condition of surgical patients. Indeed, although silver-coated Dacron is superior against bacterial growth and adherence *in vitro*, *in vivo* studies have shown similar infectivity by *S. aureus* using silver-coated and non-coated prosthesis in a porcine model of end-to-end grafting of the infrarenal aorta,[26] and silver-coated polyester failed to prevent *S. aureus* growth in a mouse model.[25] It has been suggested that the collagen fiber structure of pericardium serves as a scaffold for cellular colonization and neovascularization, which might facilitate antibiotic diffusion and response to antimicrobial therapy.[27, 28] However, whether or not porcine pericardial tubes show lower infection rates and biofilm formation *in vivo* remains to be tested. Biofilm constitution not only hampers the efficacy of antimicrobial therapies, it also allows microorganisms to escape humoral and cellular host defense mechanisms.[29]

Therefore, it will be important to address whether porcine pericardium influences *in vivo* the biofilm formation and infectivity of microorganisms, particularly yeasts.

In conclusion, stapled porcine pericardium displayed a lower susceptibility to infection by bacteria and yeasts *in vitro* when compared to the native and sutured porcine pericardium. The creation of pericardial tubes with staples is easy, rapid, and immediately sealed after declamping. Moreover, the inner surface is perfectly regular without any folding usually seen after suturing. These observations support the usage of stapled porcine pericardium over sutured pericardium to replace infected vascular grafts, especially in emergency conditions, and depending on the local preferences.

ACKNOWLEDGEMENTS

We thank Elena Santià for technical assistance

CONFLICTS OF INTEREST

None.

FUNDING

This work was supported by a grant from the Swiss National Science Foundation (SNSF) grant number 310030_173123 to TR and grant number 310030_176158 to FA.

ORCID numbers:

Thierry Roger: ORCID 0000-0002-9358-0109

Florent Allagnat: ORCID 0000-0001-6528-679X

References

1. Chakfe N, Diener H, Lejay A, Assadian O, Berard X, Caillon J, Fourneau I, Glaudemans A, Koncar I, Lindholt J*, et al.* Editor's Choice - European Society for Vascular Surgery (ESVS) 2020 Clinical Practice Guidelines on the Management of Vascular Graft and Endograft Infections. Eur J Vasc Endovasc Surg. 2020;59(3):339-84.
2. Wilson WR, Bower TC, Creager MA, Amin-Hanjani S, O'Gara PT, Lockhart PB, Darouiche RO, Ramlawi B, Derdeyn CP, Bolger AF*, et al.* Vascular Graft Infections, Mycotic Aneurysms, and Endovascular Infections: A Scientific Statement From the American Heart Association. Circulation. 2016;134(20):e412-e60.
3. Noel AA, Gloviczki P, Cherry KJ, Jr., Safi H, Goldstone J, Morasch MD, Johansen KH, United States Cryopreserved Aortic Allograft R. Abdominal aortic reconstruction in infected fields: early results of the United States cryopreserved aortic allograft registry. J Vasc Surg. 2002;35(5):847-52.
4. Dorweiler B, Neufang A, Chaban R, Reinstadler J, Duenschede F, Vahl CF. Use and durability of femoral vein for autologous reconstruction with infection of the aortoiliofemoral axis. J Vasc Surg. 2014;59(3):675-83.
5. 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(1):38-45.
6. Masmejan S, Deslarzes-Dubuis C, Petitprez S, Longchamp A, Haller C, Saucy F, Corpataux JM, Deglise S. Ten Year Experience of Using Cryopreserved Arterial Allografts for Distal Bypass in Critical Limb Ischaemia. Eur J Vasc Endovasc Surg. 2019;57(6):823-31.
7. Lejay A, Delay C, Girsowicz E, Chenesseau B, Bonnin E, Ghariani MZ, Thaveau F, Georg Y, Geny B, Chakfe N. Cryopreserved Cadaveric Arterial Allograft for Arterial Reconstruction in Patients with Prosthetic Infection. Eur J Vasc Endovasc Surg. 2017;54(5):636-44.
8. Krasznai AG, Snoeijs M, Siroen MP, Sigterman T, Korsten A, Moll FL, Bouwman LH. Treatment of aortic graft infection by in situ reconstruction with Omniflow II biosynthetic prosthesis. Vascular. 2016;24(6):561-6.
9. Topel I, Betz T, Uhl C, Wiesner M, Brockner S, Steinbauer M. Use of biosynthetic prosthesis (Omniflow II(R)) to replace infected infrainguinal prosthetic grafts--first results. Vasa. 2012;41(3):215-20.
10. 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(1):44-9.
11. Berard X, Stecken L, Pinaquy JB, Cazanave C, Puges M, Pereyre S, Bordenave L, M'Zali F. Comparison of the Antimicrobial Properties of Silver Impregnated Vascular Grafts with and without Triclosan. Eur J Vasc Endovasc Surg. 2016;51(2):285-92.
12. Czerny M, von Allmen R, Opfermann P, Sodeck G, Dick F, Stellmes A, Makaloski V, Buhlmann R, Derungs U, Widmer MK*, et al.* Self-made pericardial tube graft: a new surgical concept for treatment

of graft infections after thoracic and abdominal aortic procedures. Ann Thorac Surg. 2011;92(5):1657-62.

13. Di Marco PN, Oliver IT. Adenosine 3',5'-cyclic monophosphate and enzyme induction in the perinatal rat. FEBS Lett. 1978;94(1):183-5.

14. Kreibich M, Siepe M, Morlock J, Beyersdorf F, Kondov S, Scheumann J, Kari FA, Berger T, Schrofel H, Rylski B*, et al.* Surgical Treatment of Native and Prosthetic Aortic Infection With Xenopericardial Tube Grafts. Ann Thorac Surg. 2018;106(2):498-504.

15. Lutz B, Reeps C, Biro G, Knappich C, Zimmermann A, Eckstein HH. Bovine Pericardium as New Technical Option for In Situ Reconstruction of Aortic Graft Infection. Ann Vasc Surg. 2017;41:118-26.

16. Odero A, Argenteri A, Cugnasca M, Pirrelli S. The crimped bovine pericardium bioprosthesis in graft infection: preliminary experience. Eur J Vasc Endovasc Surg. 1997;14 Suppl A:99-101.

17. Weiss S, Tobler EL, von Tengg-Kobligk H, Makaloski V, Becker D, Carrel TP, Schmidli J, Wyss TR. Self Made Xeno-pericardial Aortic Tubes to Treat Native and Aortic Graft Infections. Eur J Vasc Endovasc Surg. 2017;54(5):646-52.

18. Yamamoto H, Yamamoto F, Ishibashi K, Chida Y, Minamiya Y, Nanjo H. In situ replacement of the thoracic aorta using an equine pericardial roll graft for an aortobronchial fistula due to aortic rupture. Gen Thorac Cardiovasc Surg. 2009;57(8):413-7.

19. Del Tatto B, Saucy F. A New Homemade Stapled Vascular Tube Graft. Eur J Vasc Endovasc Surg. 2020;59(2):320-1.

20. Roger T, Delaloye J, Chanson AL, Giddey M, Le Roy D, Calandra T. Macrophage migration inhibitory factor deficiency is associated with impaired killing of gram-negative bacteria by macrophages and increased susceptibility to Klebsiella pneumoniae sepsis. J Infect Dis. 2013;207(2):331-9.

21. Wengrovitz M, Spangler S, Martin LF. Sonication provides maximal recovery of staphylococcus epidermidis from slime-coated vascular prosthetics. Am Surg. 1991;57(3):161-4.

22. Ricco JB, 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(3):823-9.

23. Feng C, Wang H, Lu N, Chen T, He H, Lu Y, Tu XM. Log-transformation and its implications for data analysis. Shanghai Arch Psychiatry. 2014;26(2):105-9.

24. Tammer I, Reuner J, Hartig R, Geginat G. Induction of Candida albicans biofilm formation on silver-coated vascular grafts. J Antimicrob Chemother. 2014;69(5):1282-5.

25. Hernandez-Richter T, Schardey HM, Wittmann F, Mayr S, Schmitt-Sody M, Blasenbreu S, Heiss MM, Gabka C, Angele MK. Rifampin and Triclosan but not silver is effective in preventing bacterial infection of vascular dacron graft material. Eur J Vasc Endovasc Surg. 2003;26(5):550-7.

26. 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 Vasc Endovasc Surg. 2010;39(4):472-7.

27. Thampi P, Nair D, R L, N V, Venugopal S, Ramachandra U. Pathological effects of processed bovine pericardial scaffolds--a comparative in vivo evaluation. Artif Organs. 2013;37(7):600-5.

28. Mirsadraee S, Wilcox HE, Watterson KG, Kearney JN, Hunt J, Fisher J, Ingham E. Biocompatibility of acellular human pericardium. J Surg Res. 2007;143(2):407-14.

29. Arciola CR, Campoccia D, Montanaro L. Implant infections: adhesion, biofilm formation and immune evasion. Nat Rev Microbiol. 2018;16(7):397-409.

Figure Legends

Figure 1. Images of graft material used in the present study.

Figure 2. Planktonic growth and adherence of *E. coli* to graft material. Graft material was incubated at 37 °C in medium containing 10^5 CFU *E. coli*. Bacterial growth and adherence to the grafts were quantified after 6 h of incubation. Data are scatter dot plots with mean ± interquartile range. The horizontal dotted line on growth graphs indicates the inoculum at time 0. Data were analyzed using one-way ANOVA followed by Tukey's multiple comparisons test. ***$p \leq .001$; ****$p \leq .0001$; $^{\#\#\#\#}p \leq .0001$ *vs* all other groups. Full datasets of statistics are given in supplementary Tables.

Figure 3. Planktonic growth and adherence of *S. aureus* to graft material. Graft material was incubated at 37 °C in medium containing 10^5 CFU *S. aureus*. Bacterial growth and adherence to the grafts were quantified after 6 h and 24 h of incubation. Data are scatter dot plots with mean ± interquartile range. The horizontal dotted line on growth graphs indicates the inoculum at time 0. Data were analyzed using one-way ANOVA followed by Tukey's multiple comparisons test. *$p \leq .05$; **$p \leq .01$; ***$p \leq .001$; ****$p \leq .0001$. $^{\#\#\#\#}p \leq .0001$ *vs* all other groups. $^{a}p \leq .0001$ *vs.* Dacron and sutured pericardium. $^{b}p < .01$ *vs* stapled pericardium. $^{c}p < .001$ *vs.* pericardium. Full datasets of statistics are given in supplementary Tables.

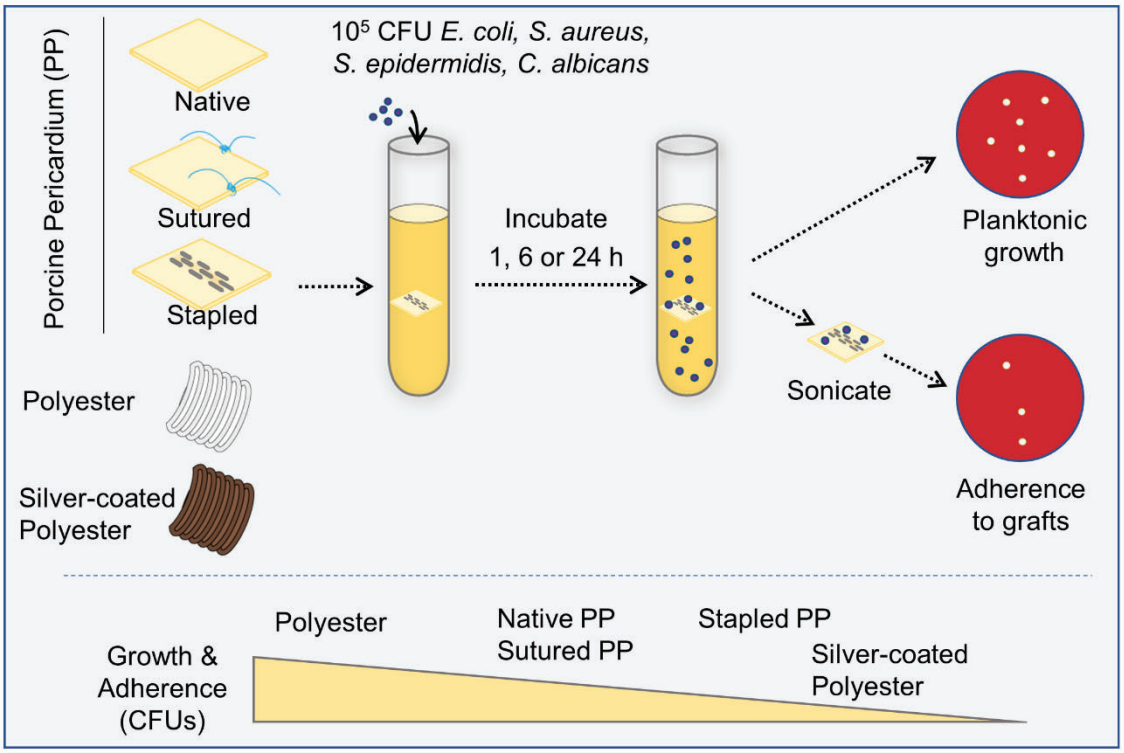
Figure 4. Planktonic growth and adherence of *S. epidermidis* to graft material. Graft material was incubated at 37 °C in medium containing 10^5 CFU *S. epidermidis*. Bacterial growth and adherence to the grafts were quantified after 6 h and 24 h of incubation. Data are scatter dot plots with mean ± interquartile range. The horizontal dotted line on growth graphs indicates the inoculum at time 0. Data were analyzed using one-way ANOVA followed by Tukey's multiple comparisons test. *$p \leq .05$; **$p \leq .01$; ***$p \leq .001$; ****$p \leq .0001$. $^{\#\#\#}p \leq .001$;

$^{\#\#\#\#}p \leq .0001$ *vs.* medium, Dacron and pericardium. $^{a}p < .001$ *vs.* medium and pericardium.$^{b}p < .01$ *vs.* Dacron and sutured pericardium. $^{c}p < .001$ *vs.* stapled pericardium. N.D.: not detected. Full datasets of statistics are given in supplementary Tables.

Figure 5. Growth of *S. aureus* and *S. epidermidis* in the presence of staples. *S. aureus* and *S. epidermidis* were incubated at 37 °C in medium containing or not staples. Bacterial growth was quantified after 6 and 24 h. Data are scatter dot plots with mean ± interquartile range.

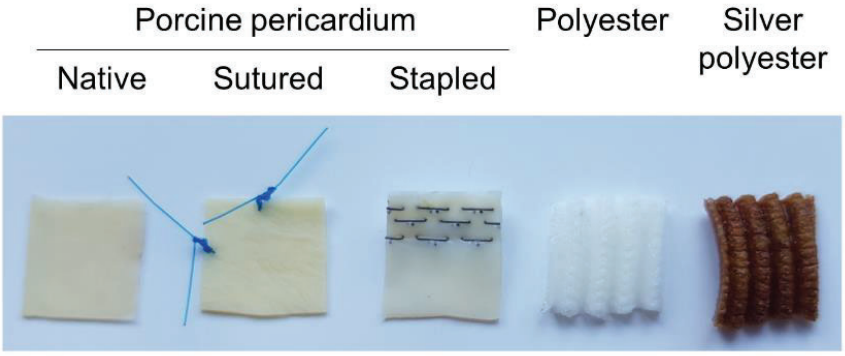
Figure 6. Planktonic growth and adherence of *C. albicans* to graft material. Graft material was incubated at 30 °C in medium containing 10^5 CFU *C. albicans*. Yeast growth and adherence to the grafts were quantified after 6 h and 24 h of incubation. Data are scatter dot plots with mean ± interquartile range. The horizontal dotted line on growth graphs indicates the inoculum at time 0. Data were analyzed using one-way ANOVA followed by Tukey's multiple comparisons test. $^{*}p \leq .05$; $^{**}p \leq .01$; $^{***}p \leq .001$; $^{****}p \leq .0001$. $^{\#\#\#\#}p \leq .0001$ *vs* all other groups. Full datasets of statistics are given in supplementary Tables.

411 **Graphical abstracts.**



412

413 **Figure 1**



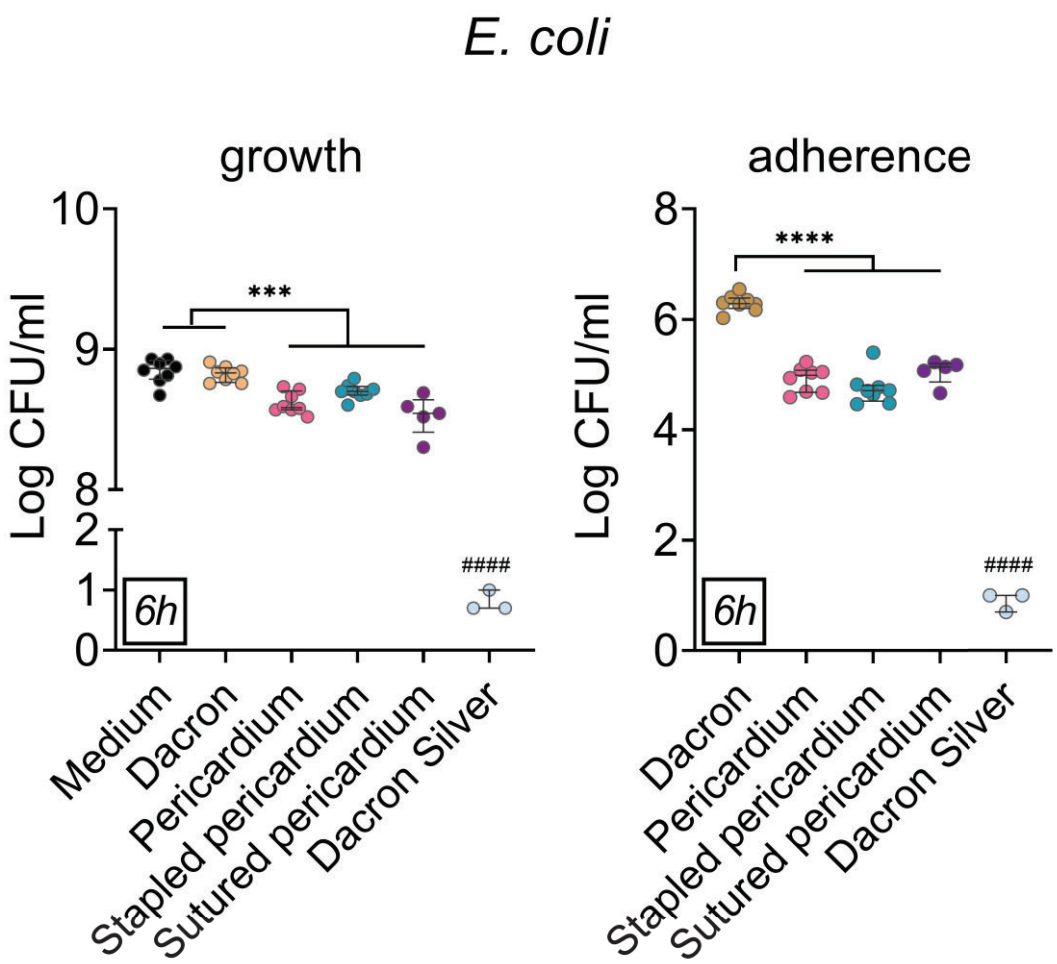
414

415

416

417 **Figure 2**

418

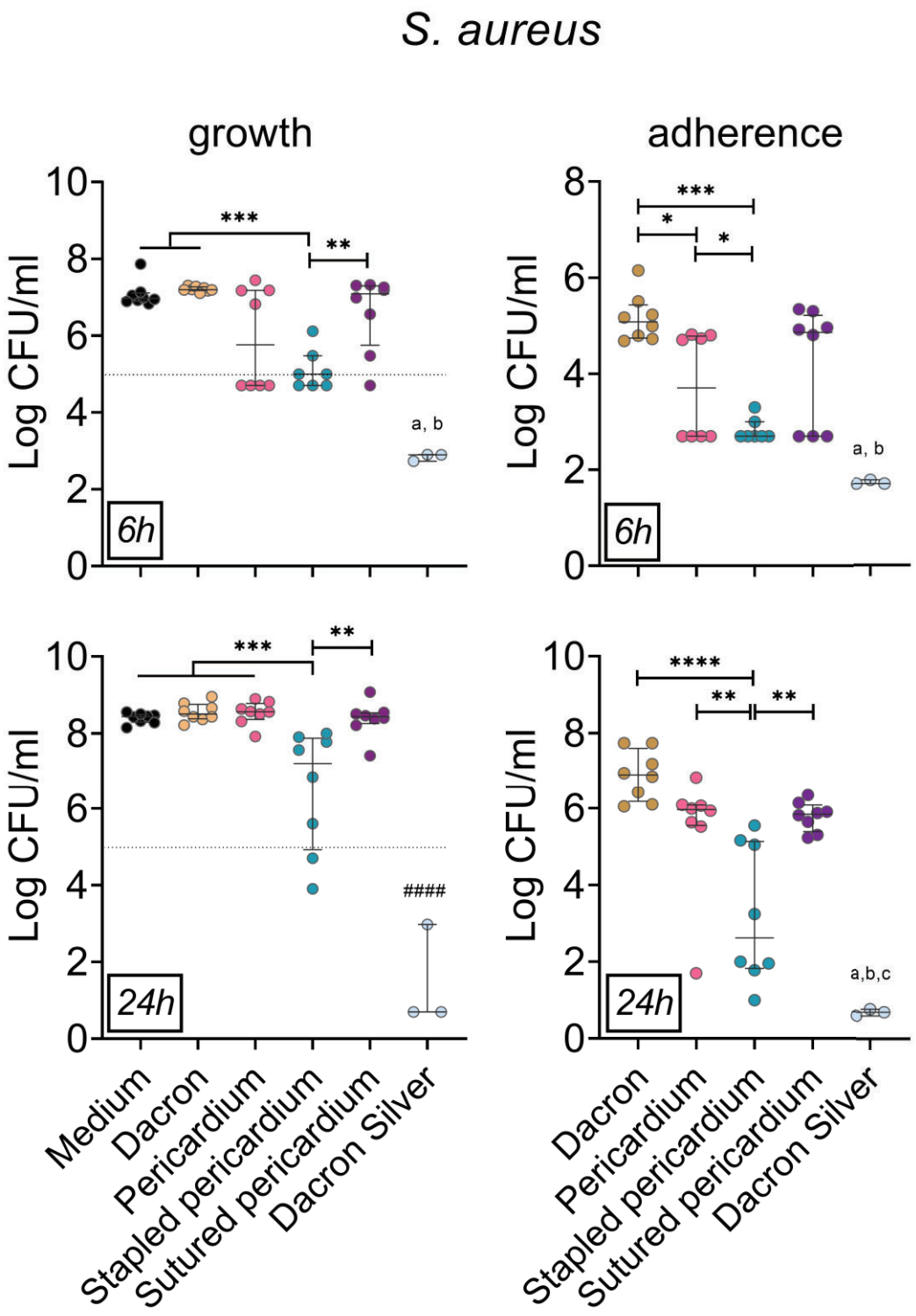


419

420

421

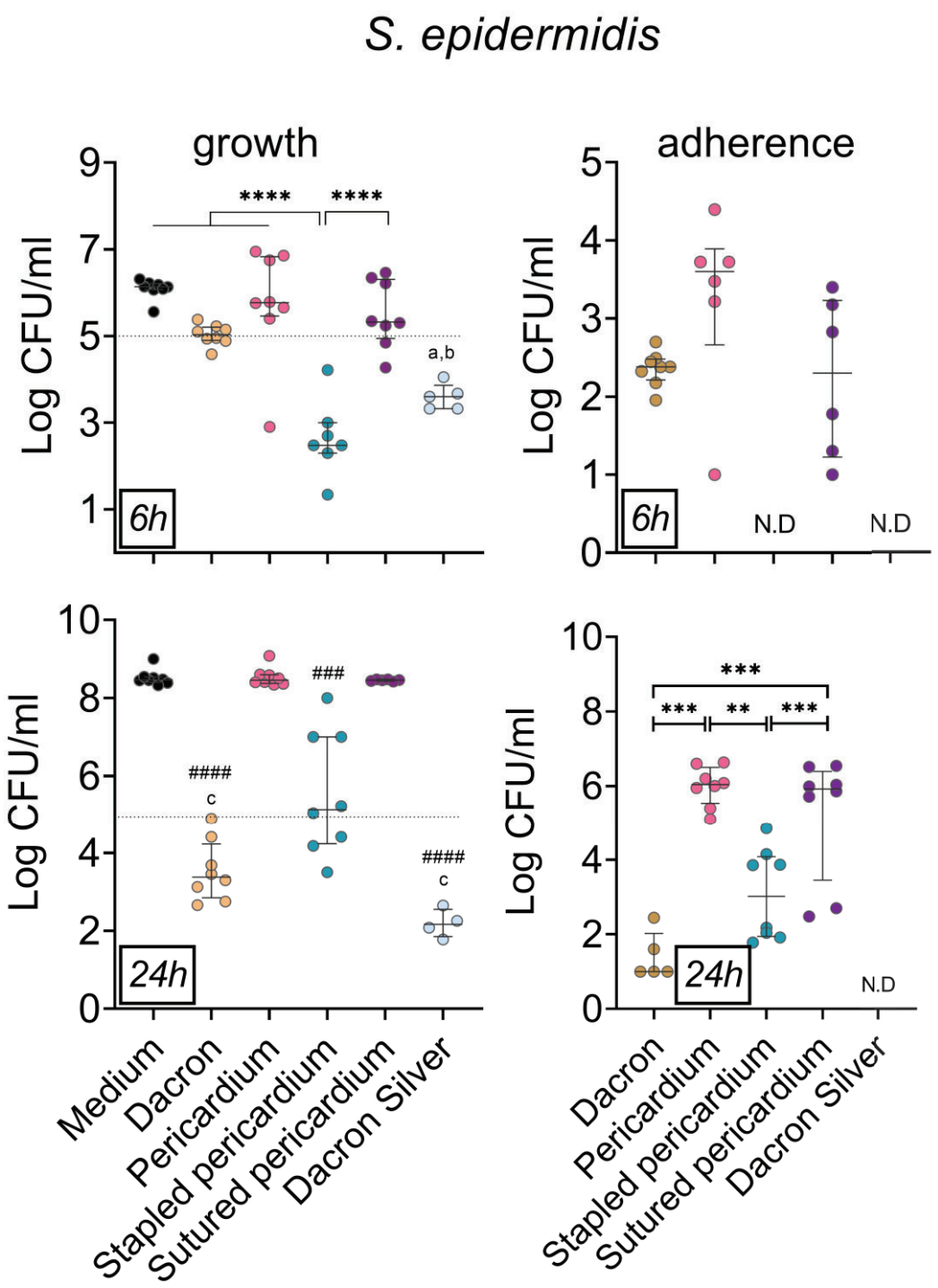
422 **Figure 3**



423

424

425 **Figure 4**



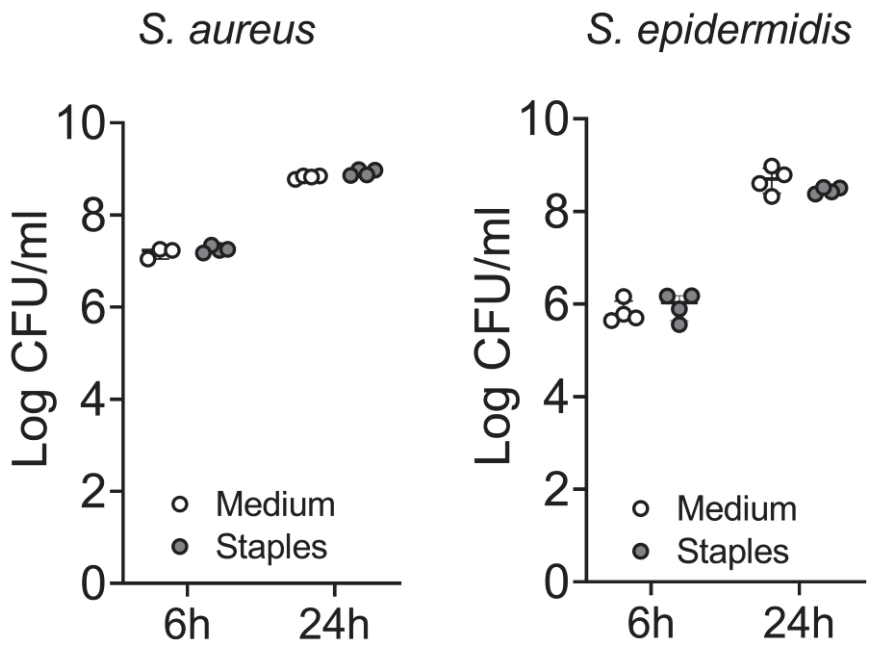
426

427

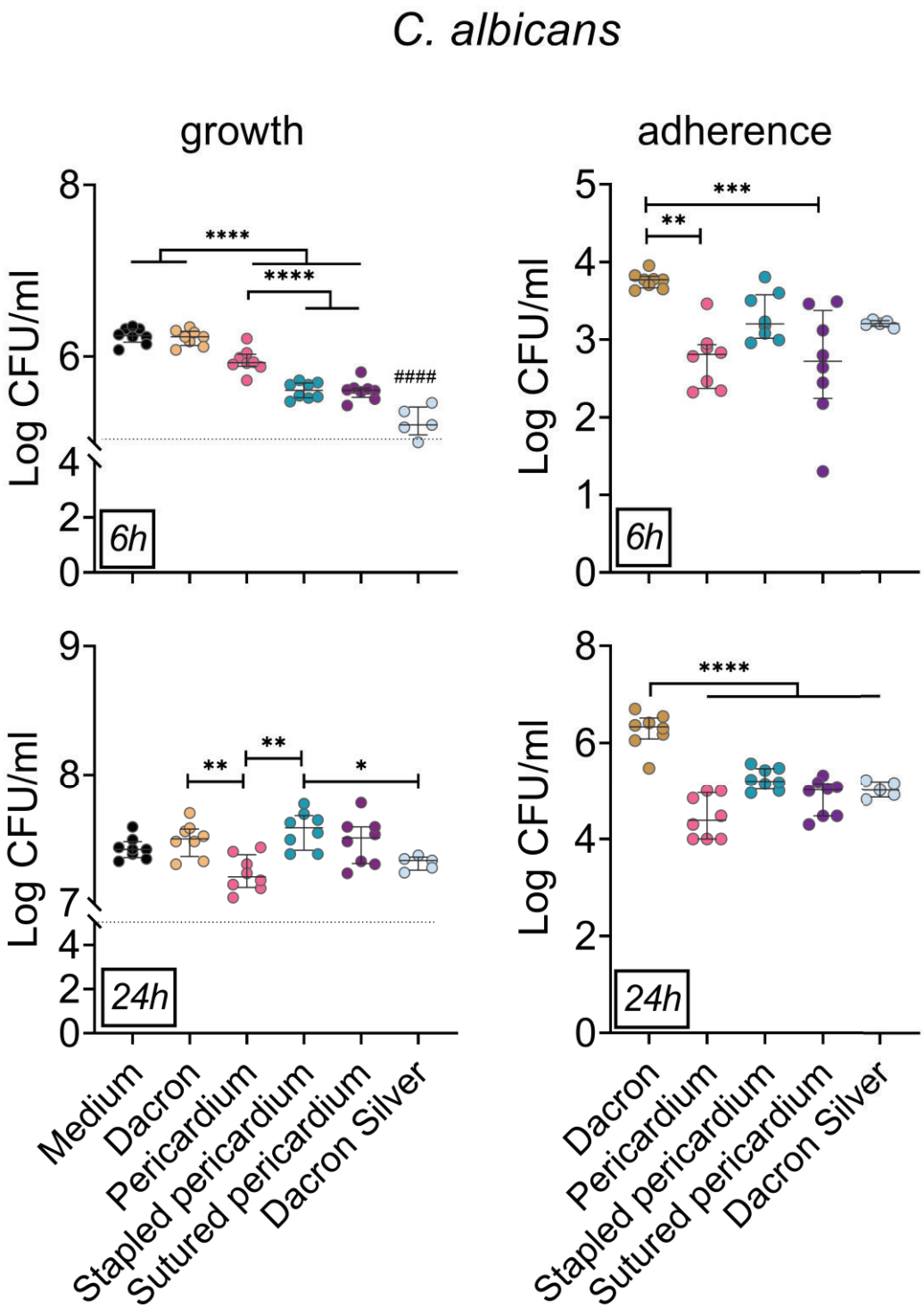
428

429

Figure 5


S. aureus
S. epidermidis
Log CFU/ml
10
8
6
4
2
0
Medium
Staples
6h
24h

435 **Figure 6**



436

437

438 **Supplementary Tables**

439

440

441 **Table S1: *E. coli* growth after 6 h of incubation**

E. coli growth	***6h***	
Tukey's multiple comparisons test	**Adjusted P Value**	
Medium vs. Pericardium	0.0002	***
Medium vs. Stapled pericardium	0.0365	*
Medium vs. Sutured pericardium	<0.0001	****
Medium vs. Dacron	0.998	ns
Medium vs. Dacron Silver	<0.0001	****
Pericardium vs. Stapled pericardium	0.4594	ns
Pericardium vs. Sutured pericardium	0.5388	ns
Pericardium vs. Dacron	0.0008	***
Pericardium vs. Dacron Silver	<0.0001	****
Stapled vs. Sutured pericardium	0.0247	*
Stapled pericardium vs. Dacron	0.0965	ns
Stapled pericardium vs. Dacron Silver	<0.0001	****
Sutured pericardium vs. Dacron	<0.0001	****
Sutured pericardium vs. Dacron Silver	<0.0001	****

442

443 **Table S2: *E. coli* adherence after 6 h of incubation**

E. coli adherence	***6h***	
Tukey's multiple comparisons test	**Adjusted P Value**	
Pericardium vs. Stapled pericardium	0.6154	ns
Per**icardium vs. Sutured pericardium**	0.8134	ns
Pericardium vs. Dacron	<0.0001	****
Pericardium vs. Dacron Silver	<0.0001	****
Stapled vs. Sutured pericardium	0.1645	ns
Stapled pericardium vs. Dacron	<0.0001	****
Stapled pericardium vs. Dacron Silver	<0.0001	****
Sutured pericardium vs. Dacron	<0.0001	****
Sutured pericardium vs. Dacron Silver	<0.0001	****
Dacron vs. Dacron Silver	<0.0001	****

444

445 **Table S3: *S. aureus* growth after 6 and 24 h of incubation**

S. aureus growth	***6h***		***24h***	
Tukey's multiple comparisons test	**Adjusted P Value**		**Adjusted P Value**	
Medium vs. Pericardium	0.054	ns	0.9994	ns
Medium vs. Stapled pericardium	0.0002	***	0.0006	***
Medium vs. Sutured pericardium	0.8163	ns	>0.9999	ns
Medium vs. Dacron	0.9993	ns	0.9987	ns
Medium vs. Dacron Silver	<0.0001	****	<0.0001	****

Pericardium vs. Stapled pericardium	0.3271	ns	0.0002	***
Pericardium vs. Sutured pericardium	0.516	ns	0.9989	ns
Pericardium vs. Dacron	0.0234	*	>0.9999	ns
Pericardium vs. Dacron Silver	<0.0001	****	<0.0001	****
Stapled vs. Sutured pericardium	0.0076	**	0.0007	***
Stapled pericardium vs. Dacron	<0.0001	****	0.0002	***
Stapled pericardium vs. Dacron Silver	0.002	**	<0.0001	****
Sutured pericardium vs. Dacron	0.6163	ns	0.9976	ns
Sutured pericardium vs. Dacron Silver	<0.0001	****	<0.0001	****
Dacron vs. Dacron Silver	<0.0001	****	<0.0001	****

446

447 **Table S4: *S. aureus* adherence after 6 and 24 h of incubation**

S. aureus adherence	***6h***		***24h***	
Tukey's multiple comparisons test	**Adjusted P Value**		**Adjusted P Value**	
Pericardium vs. Stapled pericardium	0.2746	ns	0.0068	**
Pericardium vs. Sutured pericardium	0.833	ns	0.9857	ns
Pericardium vs. Dacron	0.0188	*	0.1652	ns
Pericardium vs. Dacron Silver	0.0136	*	<0.0001	****
Stapled vs. Sutured pericardium	0.0366	*	0.0018	**
Stapled pericardium vs. Dacron	0.0001	***	<0.0001	****
Stapled pericardium vs. Dacron Silver	0.358	ns	0.0336	*
Sutured pericardium vs. Dacron	0.18	ns	0.3844	ns
Sutured pericardium vs. Dacron Silver	0.0019	**	<0.0001	****
Dacron vs. Dacron Silver	<0.0001	****	<0.0001	****

448

449 **Table S5: *S. epidermidis* growth after 6 and 24 h of incubation**

S. epidermidis growth	***6h***		***24h***	
Tukey's multiple comparisons test	**Adjusted P Value**		**Adjusted P Value**	
Medium vs. Pericardium	0.9506	ns	>0.9999	ns
Medium vs. Stapled pericardium	<0.0001	****	<0.0001	****
Medium vs. Sutured pericardium	0.6387	ns	>0.9999	ns
Medium vs. Dacron	0.079	ns	<0.0001	****
Medium vs. Dacron Silver	<0.0001	****	<0.0001	****
Pericardium vs. Stapled pericardium	<0.0001	****	<0.0001	****
Pericardium vs. Sutured pericardium	0.9841	ns	>0.9999	ns
Pericardium vs. Dacron	0.4015	ns	<0.0001	****
Pericardium vs. Dacron Silver	0.0002	***	<0.0001	****
Stapled vs. Sutured pericardium	<0.0001	****	<0.0001	****
Stapled pericardium vs. Dacron	<0.0001	****	0.0002	***
Stapled pericardium vs. Dacron Silver	0.2872	ns	<0.0001	****
Sutured pericardium vs. Dacron	0.8057	ns	<0.0001	****
Sutured pericardium vs. Dacron Silver	0.001	**	<0.0001	****
Dacron vs. Dacron Silver	0.0224	*	0.0923	ns

450

451 **Table S6: *S. epidermidis* adherence after 6 and 24 h of incubation**

S. epidermidis adherence		*6h*	*24h*	
Tukey's multiple comparisons test	**Adjusted P Value**		**Adjusted P Value**	
Pericardium vs. Stapled pericardium		nd	0.0002	***
Pericardium vs. Sutured pericardium	0.1313	ns	0.5534	ns
Pericardium vs. Dacron	0.1552	ns	<0.0001	****
Stapled vs. Sutured pericardium		nd	0.0052	**
Stapled pericardium vs. Dacron	0.9693	ns	0.0779	ns
Sutured pericardium vs. Dacron		nd	<0.0001	****

452

453 **Table S7: *C. albicans* growth after 6 and 24 h of incubation**

C. albicans growth		*6h*		*24h*
Tukey's multiple comparisons test	**Adjusted P Value**		**Adjusted P Value**	
Medium vs. Pericardium	0.0002	***	0.0613	ns
Medium vs. Stapled pericardium	<0.0001	****	0.2958	ns
Medium vs. Sutured pericardium	<0.0001	****	0.9634	ns
Medium vs. Dacron	0.9986	ns	0.9098	ns
Medium vs. Dacron Silver	<0.0001	****	0.6738	ns
Pericardium vs. Stapled pericardium	<0.0001	****	0.0002	***
Pericardium vs. Sutured pericardium	<0.0001	****	0.0076	**
Pericardium vs. Dacron	0.0006	***	0.0043	**
Pericardium vs. Dacron Silver	<0.0001	****	0.8926	ns
Stapled vs. Sutured pericardium	>0.9999	ns	0.7762	ns
Stapled pericardium vs. Dacron	<0.0001	****	0.8744	ns
Stapled pericardium vs. Dacron Silver	<0.0001	****	0.0206	*
Sutured pericardium vs. Dacron	<0.0001	****	>0.9999	ns
Sutured pericardium vs. Dacron Silver	<0.0001	****	0.2606	ns
Dacron vs. Dacron Silver	<0.0001	****	0.1887	ns

454

455 **Table S8: *C. albicans* adherence after 6 and 24 h of incubation**

C. albicans adherence		*6h*		*24h*
Tukey's multiple comparisons test	**Adjusted P Value**		**Adjusted P Value**	
Pericardium vs. Stapled pericardium	0.0924	ns	0.0006	***
Pericardium vs. Sutured pericardium	0.9959	ns	0.1566	ns
Pericardium vs. Dacron	0.0003	***	<0.0001	****
Pericardium vs. Dacron Silver	0.3326	ns	0.0468	*
Stapled vs. Sutured pericardium	0.0408	*	0.1896	ns
Stapled pericardium vs. Dacron	0.1819	ns	<0.0001	****
Stapled pericardium vs. Dacron Silver	0.9954	ns	0.8006	ns
Sutured pericardium vs. Dacron	<0.0001	****	<0.0001	****
Sutured pericardium vs. Dacron Silver	0.1937	ns	0.9092	ns
Dacron vs. Dacron Silver	0.1508	ns	<0.0001	****

456

457