EJVES Vascular Forum (2023) 59, 49–55

REVIEW

# A Narrative Review of Experimental Assessment to Study Vascular Biomaterials Infections and Infectability

**Mathilde Puges** [a,*], **Fatima M'Zali** [b], **Sabine Pereyre** [c,d], **Cécile Bébéar** [c,d], **Charles Cazanave** [a,c], **Xavier Bérard** [e]

[a] Department of Infectious and Tropical Diseases, CHU de Bordeaux, Bordeaux, France
[b] Aquitaine Microbiologie, UMR 5234 CNRS, University of Bordeaux, Bordeaux, France
[c] Mycoplasma and Chlamydia Human Infections, University of Bordeaux, USC EA 3671, Bordeaux, France
[d] Department of Bacteriology, CHU de Bordeaux, Bordeaux, France
[e] Department of Vascular Surgery, CHU de Bordeaux, Bordeaux, France

**Objective:** Many experimental studies have been conducted to evaluate vascular and endovascular graft infections (VGEIs) and infectability in order to elaborate strategies to prevent or to treat their occurrence. A systematic literature search was conducted to collect and summarise key features of infection and infectability assessment techniques in VGEI experimental models.

**Methods:** The literature search was conducted using the Medline and Cochrane databases, with no limit on the date of publication, until 10 August 2021. *Ex vivo*, *in vitro*, and *in vivo* animal studies on VGEIs, published in English or French, were selected. Cross references retrieved from selected articles on PubMed database were also included in the search. Data were collected on the techniques and the protocols performed for vascular graft infection and infectability assessment.

**Results:** A total of 243 studies were included in the review: 55 *in vitro* studies, 169 animal studies, 17 combining the two models, and two *ex vivo* studies. Many experimental techniques were performed, with a lot of protocol discrepancies. The main experiments conducted were bacterial culture, with ($n = 82$ studies) or without sonication ($n = 120$), histopathology ($n = 69$), scanning electron microscopy ($n = 36$), and graft diffusion tests ($n = 28$). These techniques were used to answer different research questions corresponding to different graft infection steps, such as microbial adhesion and/or viability, biofilm biomass or organisation, human cell reaction, or antimicrobial activity.

**Conclusion:** Many experimental tools are available to study VGEIs, but to improve their reproducibility and scientific reliability research protocols must be standardised and include sonication of grafts before microbiological culture. Moreover, the key role of the biofilm in VGEI physiopathology must be taken into account in future studies.



Article history: Received 5 April 2022, Revised 23 March 2023, Accepted 9 May 2023,

**Keywords:** Biofilm, Infectability, Review, Vascular biomaterials, Vascular graft and endograft infections

## INTRODUCTION

Vascular graft and endograft infections (VGEIs) are still associated with high morbidity, mortality, and relapse rates,[1] but their physiopathology and treatment remain insufficiently understood. Two major issues are addressed in VGEI experimental studies: the infectability and the infection. Graft infectability is the susceptibility of a vascular biomaterial to be infected, while graft infection refers to a later stage, when microorganisms have already adhered and colonised the biomaterial. These two terms represent successive steps of VGEIs but do not respond to the same research issues.

The issue of infectability of vascular biomaterials is of particular importance to improve the outcome of VGEIs and to decrease their occurrence and their relapse on new implanted grafts. However, even the word "infectability" is not clearly defined in the literature. The first occurrence of this word appears in 1975 in an experimental study, defined as "that the susceptibility of a prosthetic graft to infection";[2] however, no consensual definition is currently available in the literature. This first definition gives rise to multiple notions of what susceptibility to infection can be. Indeed, microbial colonisation of a biomaterial surface implies several crucial steps that involve different pathophysiological mechanisms, and infectability assessment should be considered through these different mechanisms.

* Corresponding author. Department of Infectious and Tropical Diseases, CHU de Bordeaux, F-33000, Bordeaux, France.
*E-mail address:* mathilde.puges@u-bordeaux.fr (Mathilde Puges).



https://doi.org/10.1016/j.ejvsvf.2023.05.002

Studies on graft infection focus on biofilm formation, anti-infectious and antibiofilm treatments, surgical strategies, and microbiological and imaging diagnosis. These two notions are therefore part of a continuum, since the infectability reflects the susceptibility to infection.

Two recent well conducted reviews have been published on preclinical models evaluating vascular graft coating and silver grafts,[3,4] but no review has been conducted so far on the analysis performed in experimental models to assess vascular graft infectability and infection. This review was conducted to collect and summarise key features of infection and infectability assessment techniques in VGEI experimental models. The second aim was to classify these techniques according to different research issues. This work is complementary to a previous review published by this multidisciplinary team on experimental models of VGEIs.[5]

## MATERIAL AND METHODS

The study was conducted in accordance with the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-analysis) guidelines[6] and the PICO strategy (Patient Population [P], Intervention [I], Comparison [C], and Outcomes [O]), which were used to structure and respond to the research question. PICO criteria were: "In *in vitro* and *in vivo* models of VGEIs (P), which experimental techniques (I) are the most accurate (C) to investigate the issues on vascular graft infection and infectability (O)"?

### *Search strategy and information sources*

A duplicate comprehensive literature hand search was conducted by M.P. and C.C. using the Medline and Cochrane databases, with no limit on the date of publications. Disagreements between M.P. and C.C. were resolved by consensus. If consensus was not achievable, a third opinion was given by X.B. The search was updated on 10 August 2021. The search terms for Medline and the Cochrane Database search were as follows: "vascular graft infection" or "vascular graft infectability" or "vascular graft infectibility" combined using the Boolean operator "AND" with "animal study", "animal model", "in vitro study", or "in vitro model". The MeSH terms "blood vessel prosthesis" AND "infections" AND "models, animal", and "blood vessel prosthesis" AND "infections" AND "in vitro techniques" were also used. For each included article, the reference list and the first 20 related articles on PubMed were screened to retrieve potentially relevant articles.

### *Eligibility criteria and study selection*

Studies were selected according to the following criteria. Only *in vitro* and *in vivo* animal studies on VGEIs, published in English or French, were selected. Clinical studies on human beings and experimental studies on other device infections were excluded from analysis. Studies were first selected on the basis of title and abstract, then on full text. Duplicates were discarded.

### *Data collection process*

Data were extracted by M.P. and C.C. From each study, the source (main author, journal, and year of publication) and the details the analysis performed for infectability and/or infection assessment were collected.

## RESULTS

A total of 243 articles were included in the narrative review: 55 in vitro studies, 169 animal studies, 17 which combined both models, and two ex vivo studies (Fig. 1). The most employed and/or promising techniques performed were study infection and infectability, and their purposes were successively detailed. More anecdotal analysis (seldomly and/or formerly used) are detailed in Supplementary Data 1. All techniques are summarised in Supplementary Table 1 and illustrated in Fig. 2.

### *Microbial culture*

The microbial count in colony forming units (CFU)/mL is often considered as the gold standard for the quantification of bacterial adherence on vascular graft material and is the most used analysis, in both *in vitro* and *in vivo* studies. The other tests were more seldomly used (Supplementary Data 1).

**Microbial count on infected grafts.** In most studies, grafts and perigraft tissues were processed using different techniques before plating, in order to enhance bacterial culture sensitivity. Grafts were sometimes sonicated before culture ($n = 82$ studies), sometimes associated with vortexing,[7] but the sonication process varied a lot among studies. Its length varied between 1 minute and 20 minutes, but most often was for 5 minutes.[8] Ultrasound frequency was rarely specified and varied between 20 kHz and 60 KHz. Sonication efficacy was assessed in several studies either by scanning electron microscopy (SEM)[9] or optical microscopy.[10] However, most studies did not used sonication ($n = 120$).

Sometimes, grafts were only vigorously agitated[11] or vortexed 30 seconds to several minutes, until 1 hour of vortexing.[12] Other techniques were used, such as homogenisation with a dispersing instrument,[13] ultrasonic disruption,[14] bead beaters with glass beads,[15] hand crushing with a sterile mortar,[16] tissue grinding associated with sonication,[17] or trypsin digestion of the graft and perigraft tissues.[18]

However, in several studies, grafts were not processed before culture but only placed in broth medium before plating on agar.[19] Sometimes grafts were only rolled on agar and removed before culture,[20] or only imprinted on agar before incubation.[21]

Despite protocol discrepancies, microorganism recovery was better with sonication than with vortexing alone[14] or ultrasonic disruption.[14] This superiority in bacterial recovery has been assessed in both *in vitro*[14,22] and animal models,[17,23] especially for *Staphylococcus epidermidis*,[14,17,22,23] but also *Staphylococcus aureus*,

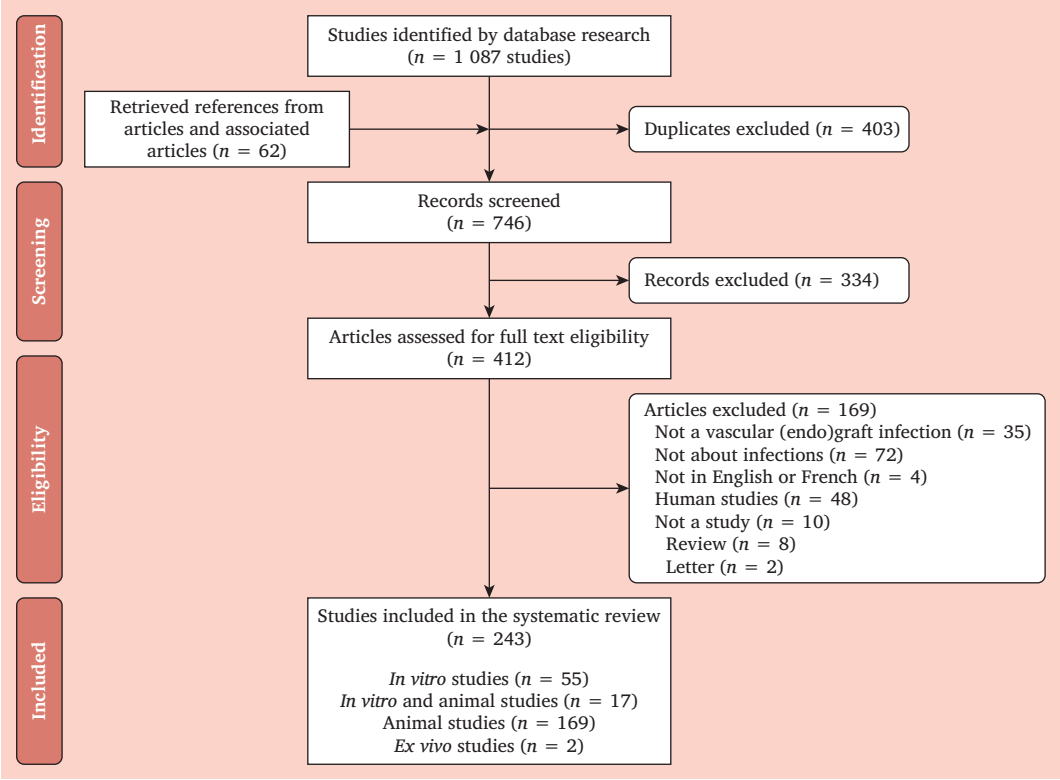


**Fig. 1.** Flow chart according to Preferred Reporting Items for Systematic Reviews and Meta-analysis guidelines.

*Escherichia coli*, *Enterococcus faecalis*, and *Klebsiella pneumoniae*.[14]

The first animal study published in 1989 by Bergamini et al. demonstrated an enhanced recovery of *S. epidermidis* on aortic replacement grafts in dogs after sonication compared with broth culture alone (8/36 vs 0/36, $p < .010$).[17] In another animal study conducted on dogs, sonication also significantly improved the bacterial culture positivity rate of *S. epidermidis* ($p < .010$).[23] *In vitro* studies demonstrated a higher bacterial load recovered from the graft after sonication than ultrasonic disruption or vortexing (>1 log).[14,22]

Among all these techniques evaluated to enhance microbial culture sensitivity, sonication was the most employed and studied one, and therefore seems to be the most reliable technique. Indeed, sonication with adapted parameters helps to dislodge biofilm and viable microorganisms from the graft surface.

**Kill kinetic studies (n = 8 studies).** By repeating the bacterial count in the supernatant at several times of culture, this analysis was meant to study either the impact and the efficacy of several antibiotic molecules, or graft infectability and bactericidal activity of antimicrobial grafts.[24] A bactericidal effect was defined as a $\geq 3$ $\log^{10}$ reduction in CFU compared with the initial inoculum.[25]

Thanks to the evaluation of bacterial count at successive time slots, this technique is particularly useful to evaluate the efficacy of antimicrobial molecules and grafts over time and biofilm formation.

### *Viability assessments*

**Adenosine triphosphate assay (n = 2 studies).** The mitochondrial adenosine triphosphate (ATP) concentration, directly proportional to the amount of ATP in bacteria, was measured to quantify the number of viable bacteria. This test has also been used in these studies to compare several antimicrobial molecules *in vitro* but could be useful to estimate the bacterial inoculate embedded in biofilm on graft surface with no need to dislodge this biofilm.

**Biofilm bacterial metabolism assays (n = 4 studies).** This colorimetric assay is based on the reduction of a yellow tetrazolium salt to purple formazan crystals by the mitochondrial dehydrogenase of metabolically active cells. This semi-quantitative test, also called MTT or XTT, has notably been used *in vitro* to evaluate metabolic activity of microorganisms in vascular graft biofilm. In the same way as the ATP assay, this could also be an interesting technique to assess viable bacterial load in biofilm of grafts.

### *Antimicrobial activity in biofilm*

**Minimal biofilm inhibitory concentrations and minimum biofilm eliminating concentrations determination (n = 7 studies).** Minimal biofilm inhibitory concentrations (MBICs) and minimum biofilm eliminating concentrations (MBECs) were often further compared with minimum inhibitory concentration (MIC). Before the concept of MBIC and MBEC, some authors determined both MIC and minimal bactericidal concentration (MBC) using the tube

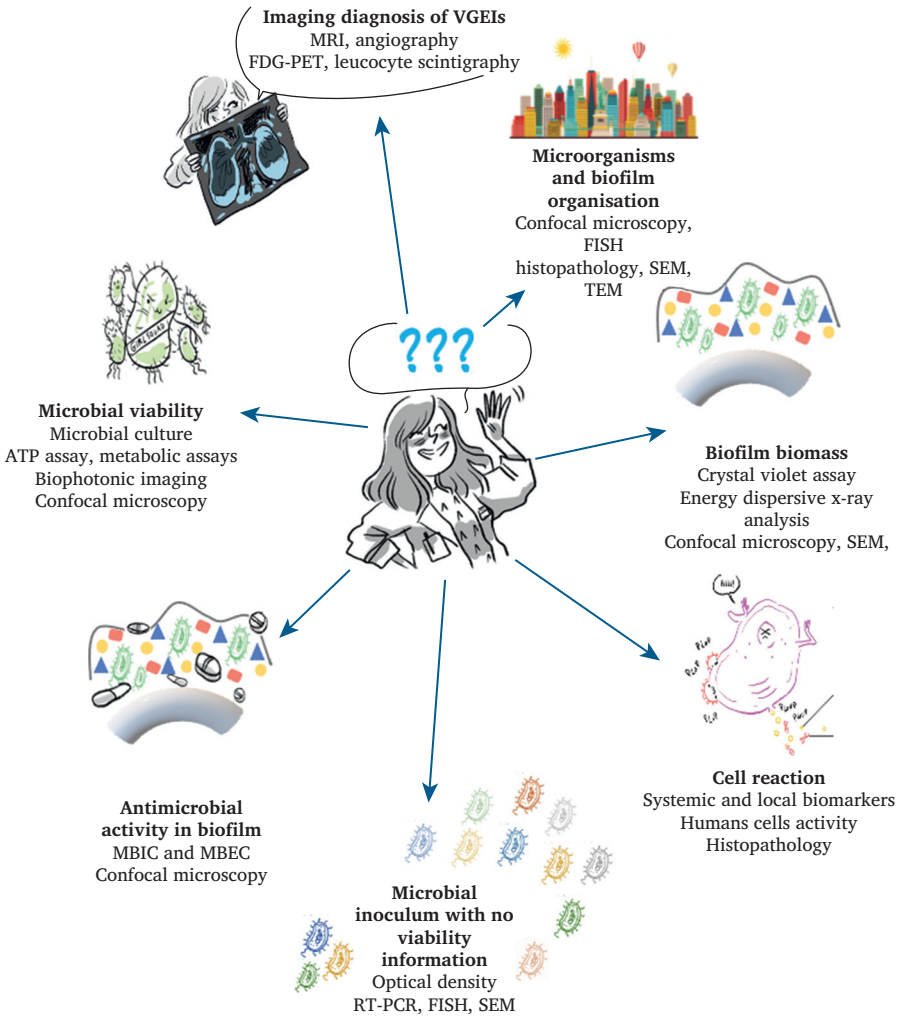


**Fig. 2.** Experimental analysis relevant to answer selective research questions. VGEI, vascular and endovascular graft infection; ATP = adenosine triphosphate; MBIC = minimal biofilm inhibitory concentration; MBEC = minimum biofilm eliminating concentration; RT-PCR = reverse transcriptase polymerase chain reaction; SEM = scanning electron microscopy; TEM = transmission electron microscopy; MRI = magnetic resonance imaging; FDG-PET = fluorodeoxyglucose-positron emission tomography; FISH: fluorescence *in situ* hybridisation.

microdilution method or by the agar dilution method. The impact on MIC and MBC of antimicrobial molecules has also been studied.

These concentrations should be performed experimentally to evaluate the actual activity of antimicrobial molecules in biofilm.

### *Biofilm staining*

**Crystal violet assay (n = 3 studies).** The crystal violet (CV) assay uses a basic dye that binds negatively charged molecules and thus stains both bacteria and the surrounding biofilm matrix.[26] This technique was particularly used in *vitro* studies but also *in vivo* studies to characterise the bacterial strain before the onset of infection in animal models. Only one study reported that the measurement of CV via spectrophotometry was not suitable for vascular graft surfaces because of high unspecific background staining.[27] However, two other studies have been published since this first observation, to evaluate biofilm formation in contact with a graft coating agent. The walls of microplate well were covered either with gelatin, collagen, or an antibiotic containing fibrin gel before bacterial inoculation, culture, and then CV assay. CV assay might lack specificity and allows no distinction in biofilm components; however, its protocol is well standardised and easy to achieve. Moreover, its frequent use in previous studies better allows comparison of results (Supplementary Data 1).

### *Microscopy*

**Scanning electron microscopy (n = 36 studies).** SEM has been used to better understand VGEI physiopathology, notably biofilm formation and microorganism adhesion and organisation on the graft surface. Rarely, SEM results were analysed to estimate the approximate number of adhesive bacterial microcolonies on graft segments. SEM has also been used to observe biofilm formation kinetics on the graft surface by serial acquisitions, graft endothelialisation, and susceptibility to infection. Some studies tried to assess the

sonication efficacy on dislodging bacteria by SEM, or the efficacy of several antimicrobial treatments or antiseptic molecules on infected vascular grafts. Only one *ex vivo* study used SEM to analyse explanted infected grafts from patients operated on for VGEI.[28] SEM can still be useful to study biofilm conformation on infected grafts, notably to visualise the effects of antimicrobial therapy and/or grafts (Supplementary Data 1).

**Confocal microscopy (n = 8 studies).** Confocal microscopy was first applied by Osada et al. in 1999 to study and compare bacterial adhesion.[29] *In vitro*, confocal microscopy has been used to assess the thickness, density, and formation kinetics of biofilm on various grafts with different dyes. In animal models, confocal microscopy has been applied to localise bacteria, immune cells, and antibiotics with specific antibodies marked with fluorochromes. Confocal microscopy and fluorochrome dyeing protocols are well standardised and resolution is constantly improving. Moreover, they allow the analysis of microorganisms and host cells and their interaction.

### *Imaging*

**In vivo bioluminescence imaging (n = 1 study).** This non-invasive imaging technique was evaluated only once in VGEIs. The authors inoculated a bioluminescent *S. aureus* strain, genetically engineered in order to express a modified *Photorhabdus* luminescence lux operon, on vascular grafts subcutaneously implanted in mice backs. The real time bioluminescence was correlated with the total number of bacteria to estimate the bacterial burden.[30] This technique could be interesting to study the dynamics of microbial adhesion and biofilm formation in VGEIs (Supplementary Data 1).

## DISCUSSION

A narrative review was conducted on 243 studies evaluating infectability and infection assessment analysis in VGEI experimental models. Many interesting techniques have been used to answer various research questions on vascular graft infectability and infection, and future researchers should select the analysis to perform according to their hypotheses. All the analyses performed were summed up for infection and infectability assessment in experimental models of VGEIs (Fig. 2). To date, most published studies have used microbial culture and/or microscopy to answer their research issues.

In the following paragraphs, the focus will be on three main points of interest: the necessity of protocol standardisation and particularly the importance of sonication before microbial culture, the need for techniques which take into account adhesion and biofilm formation, and finally the potential contribution of new non-invasive techniques.

Infectability and infection assessment techniques need standardisation to be relevant and applicable to clinical practice. Indeed, experimental protocols varied a lot among studies, especially for microbial culture. For instance, in most studies, which appeared to be the earliest ones, infected vascular grafts were not sonicated. The better efficacy for microbial recovery after sonication has been assessed in several *in vitro* and *in vivo* studies on VGEIs.[14,17,22,23] Its clinical impact on VGEI microbiological diagnosis has been well demonstrated in bone and joint infections (BJIs)[31] and remains likely in VGEIs.[32] It should therefore be used in experimental studies before bacterial count on grafts. However, the sonication protocol needs standardisation, and here a protocol is proposed based on the most employed protocols in the previously published studies, which might offer the best microbial recovery along with maximal viability. Grafts should be processed as follows: vortexing for 30 seconds, sonication for 5 minutes at a ultrasonic frequency between 35 and 50 kHz, and then vortexing for another 30 seconds before sonicate fluid plating.[8,31,32] Finally, to help standardisation in future studies, researchers should adopt the protocols previously published, and, if not, they should explain why they chose to change a particular step or reagent. Moreover, there might be room for Delphi consensus on selected topics in the VGEI experimental research field.

Furthermore, some issues remain underexplored in the VGEI field, especially microbial adhesion and biofilm. In the vast majority of studies, graft infectability experiments did not include any analysis of biofilm. Investigations on antimicrobial therapy in experimental models of VGEIs are rare, particularly regarding their activity in the presence of VGEI biofilm. More importantly, no particular attention was given to the ability of antimicrobials to penetrate biofilm and to differentiate antimicrobial activity in the presence of an established biofilm. Bacterial adherence and biofilm formation on grafts are certainly key steps to understand the onset of VGEIs and infection relapses. Some very interesting studies have been recently published using confocal microscopy to analyse bacterial biofilm formation on different grafts.[8] Confocal microscopy has also allowed the recent assessment that biofilm structures were correlated with antibiotic activity, and that *S. aureus* was able to survive inside macrophages. In the same study, the authors also found that neither daptomycin nor vancomycin were able to penetrate macrophages.[33] These recent findings have allowed great progress in the understanding of VGEIs and have opened up a whole new area for VGEI research, where VGEI physiopathology needs to be re-interpreted through the lens of biofilm and immune cells. Indeed, this intracellular bacterial persistence has already been described in BJIs. *S. aureus* and *S. epidermidis* can persist in acidic phagolysosomes of osteoblasts and fibroblasts, partly as small colony variants, and that might contribute to chronic BJI relapses.[34] They further identified antibiotics with intracellular activities which should be preferred in BJI therapy in order to eradicate this lysosomal reservoir of bacteria.[34] In any case, research on biofilm formation, biofilm characteristics, and the best antimicrobials active in biofilm is far more advanced in the BJI field. However, findings on BJI biofilms cannot be directly extrapolated to

VGEIs due to many differences between these two types of infection, including cell environment, blood flow, and high shear stress applied to vascular grafts. Even the microorganisms are different, and polymicrobial infections are more frequent in VGEIs, especially in aortic position. Moreover, confocal microscopy applications are numerous and promising, since new microscopes notably allow graft cartography, live imaging, and visualisation of microbial interactions in biofilm along with interactions between host cells and microorganisms. These new applications could be of a great interest to better elucidate VGEI physiopathology and advocate for a larger use of confocal microscopy in future studies.

New techniques that allow *in vivo* imaging, such as bioluminescence and fluorescent imaging technologies, also appear of particular interest, and are mentioned as research priorities in the recently published European consensus[1] and should be promoted. These techniques offer the opportunity to observe the *in vivo* course of biomaterial associated infections in small animals, without the need to sacrifice animals at different time points after the onset of infection,[35] and could be useful for the *in vivo* evaluation of antimicrobial treatments and/or antimicrobial graft coating effects on bacterial biofilm.[35] Only Lorenz et al. have studied bioluminescent imaging in VGEIs.[30] This approach has also been evaluated in animal models of BJIs[36] and vascular catheters.[37] Other promising non-invasive techniques have been evaluated in VGEIs, such as magnetic resonance imaging and positron emission tomography (PET) computed tomography,[38] or in vascular catheter infections with fluorodeoxyglucose-microPET.[39] Finally, alternative technologies to avoid animal testing, such as *ex vivo* flow models, which have already been experimented on the VGEI field, but also computed simulation, will hopefully increase in the coming years.[40]

Hopefully this review will support the need for protocol standardisation in order to help comparability of experimental studies, but also the importance of selecting accurate techniques to get closer to the truth about vascular biomaterial infectability and infection.

### *Conclusion*

Experimental research on VGEIs needs standardisation and reproducibility. Many techniques have been employed over the years but discrepancies in research protocols do not allow their comparison. The most striking example is sonication, which is still poorly used in VGEI studies, despite a proven utility for improving microbiological culture performance. Finally, the key role of biofilm in VGEI physiopathology must be taken into account in future studies.

## CONFLICT OF INTEREST

None.

## FUNDING

None.

## APPENDIX A. SUPPLEMENTARY DATA

Supplementary data related to this article can be found at https://doi.org/10.1016/j.ejvsvf.2023.05.002.

## REFERENCES

1 Chakfé N, Diener H, Lejay A, Assadian O, Berard X, Caillon 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**:339—84.

2 Malone JM, Moore WS, Campagna G, Bean B. Bacteremic infectability of vascular grafts: the influence of pseudointimal integrity and duration of graft function. *Surgery* 1975;**78**:211—6.

3 Mufty H, Van Den Eynde J, Meuris B, Metsemakers WJ, Van Wijngaerden E, Vandendriessche T, et al. Pre-clinical in vivo models of vascular graft coating in the prevention of vascular graft infection: a systematic review. *Eur J Vasc Endovasc Surg* 2021;**62**:99—118.

4 Mufty H, Van den Eynde J, Steenackers HP, Metsemakers WJ, Meuris B, Fourneau I. A systematic review of preclinical data regarding commercial silver-coated vascular grafts. *J Vasc Surg* 2021;**74**:1386—93.

5 Puges M, M'Zali F, Pereyre S, Bébéar C, Cazanave C, Bérard X. A narrative review of experimental models to study vascular grafts infections. *EJVES Vasc Forum* 2022;**55**:30—7.

6 Moher D, Shamseer L, Clarke M, Ghersi D, Liberati A, Petticrew M, et al. Preferred reporting items for systematic review and meta-analysis protocols (PRISMA-P) 2015 statement. *Syst Rev* 2015;**4**:1.

7 Kirkton RD, Prichard HL, Santiago-Maysonet M, Niklason LE, Lawson JH, Dahl SLM. Susceptibility of ePTFE vascular grafts and bioengineered human acellular vessels to infection. *J Surg Res* 2018;**221**:143—51.

8 Revest M, Jacqueline C, Boudjemaa R, Caillon J, Le Mabecque V, Breteche A, et al. New in vitro and in vivo models to evaluate antibiotic efficacy in Staphylococcus aureus prosthetic vascular graft infection. *J Antimicrob Chemother* 2016;**71**:1291—9.

9 Schmitt DD, Bandyk DF, Pequet AJ, Towne JB. Bacterial adherence to vascular prostheses. A determinant of graft infectivity. *J Vasc Surg* 1986;**3**:732—40.

10 Balaban N, Giacometti A, Cirioni O, Gov Y, Ghiselli R, Mocchegiani F, et al. Use of the quorum-sensing inhibitor RNAIII-inhibiting peptide to prevent biofilm formation in vivo by drug-resistant Staphylococcus epidermidis. *J Infect Dis* 2003;**187**:625—30.

11 Benvenisty AI, Tannenbaum G, Ahlborn TN, Fox CL, Modak S, Sampath L, et al. Control of prosthetic bacterial infection: evaluation of an easily incorporated, tightly bound, silver antibiotic PTFE graft. *J Surg Res* 1988;**44**:1—7.

12 Staneviciute E, Na'amnih W, Kavaliauskas P, Prakapaite R, Ridziauskas M, Kevlicius L, et al. New in vitro model evaluating antiseptics' efficacy in biofilm-associated Staphylococcus aureus prosthetic vascular graft infection. *J Med Microbiol* 2019;**68**:432—9.

13 Muhl E, Gatermann S, Iven H, Dendorfer A, Bruch HP. Local application of vancomycin for prophylaxis of graft infection: release of vancomycin from antibiotic-bonded Dacron grafts, toxicity in endothelial cell culture, and efficacy against graft infection in an animal model. *Ann Vasc Surg* 1996;**10**:244—53.

14 Padberg FT, Smith SM, Eng RH. Optimal method for culturing vascular prosthetic grafts. *J Surg Res* 1992;**53**:384—90.
15 Javerliat I, Goëau-Brissonnière O, Sivadon-Tardy V, Coggia M, Gaillard JL. Prevention of Staphylococcus aureus graft infection by a new gelatin-sealed vascular graft prebonded with antibiotics. *J Vasc Surg* 2007;**46**:1026—31.
16 Coggia M, Goëau-Brissonnière O, Leflon V, Nicolas MH, Pechère JC. Experimental treatment of vascular graft infection due to Staphylococcus epidermidis by in situ replacement with a rifampin-bonded polyester graft. *Ann Vasc Surg* 2001;**15**:421—9.
17 Bergamini TM, Bandyk DF, Govostis D, Vetsch R, Towne JB. Identification of Staphylococcus epidermidis vascular graft infections: a comparison of culture techniques. *J Vasc Surg* 1989;**9**:665—70.
18 Henke PK, Bergamini TM, Watson AL, Brittian KR, Powell DW, Peyton JC. Bacterial products primarily mediate fibroblast inhibition in biomaterial infection. *J Surg Res* 1998;**74**:17—22.
19 Lachapelle K, Graham AM, Symes JF. Antibacterial activity, antibiotic retention, and infection resistance of a rifampin-impregnated gelatin-sealed Dacron graft. *J Vasc Surg* 1994;**19**:675—82.
20 Fletcher JP, Dryden M, Munro R, Xu JH, Hehir MD. Establishment of a vascular graft infection model in the sheep carotid artery. *Aust NZ J Surg* 1990;**60**:801—3.
21 Richardson RL, Pate JW, Wolf RY, Ledes C, Hopson WB. The outcome of antibiotic-soaked arterial grafts in Guinea pig wounds contaminated with E. coli or S. aureus. *J Thorac Cardiovasc Surg* 1970;**59**:635—7.
22 Wengrovitz M, Spangler S, Martin LF. Sonication provides maximal recovery of staphylococcus epidermidis from slime-coated vascular prosthetics. *Am Surg* 1991;**57**:161—4.
23 Tollefson DF, Bandyk DF, Kaebnick HW, Seabrook GR, Towne JB. Surface biofilm disruption. Enhanced recovery of microorganisms from vascular prostheses. *Arch Surg* 1987;**122**:38—43.
24 Berard X, Puges M, Pinaquy JB, Cazanave C, Stecken L, Bordenave L, et al. In vitro Evidence of improved antimicrobial efficacy of silver and triclosan containing vascular grafts compared with rifampicin soaked grafts. *Eur J Vasc Endovasc Surg* 2019;**57**:424—32.
25 Giacometti A, Cirioni O, Ghiselli R, Goffi L, Mocchegiani F, Riva A, et al. Efficacy of polycationic peptides in preventing vascular graft infection due to Staphylococcus epidermidis. *J Antimicrob Chemother* 2000;**46**:751—6.
26 Stepanović S, Vuković D, Dakić I, Savić B, Švabić-Vlahović M. A modified microtiter-plate test for quantification of staphylococcal biofilm formation. *J Microbiol Methods* 2000;**40**:175—9.
27 Herten M, Bisdas T, Knaack D, Becker K, Osada N, Torsello GB, et al. Rapid in vitro quantification of S. aureus biofilms on vascular graft surfaces. *Front Microbiol* 2017;**8**:2333.
28 Chakfe N, Guidoin R, Marois M, Roy PE, Douville Y, Roy P, et al. A pathological study of arterial prostheses surgically excised after overt clinical infection. *J Biomater Appl* 1991;**5**:227—46.
29 Osada T, Yamamura K, Fujimoto K, Mizuno K, Sakurai T, Ohta M, et al. Prophylaxis of local vascular graft infection with levofloxacin incorporated into albumin-sealed Dacron graft (LVFX-ALB Graft). *Microbiol Immunol* 1999;**43**:317—21.
30 Lorenz U, Schäfer T, Ohlsen K, Tiurbe GC, Bühler C, Germer CT, et al. In vivo detection of Staphylococcus aureus in biofilm on vascular prostheses using non-invasive biophotonic imaging. *Eur J Vasc Endovasc Surg* 2011;**41**:68—75.
31 Trampuz A, Piper KE, Jacobson MJ, Hanssen AD, Unni KK, Osmon DR, et al. Sonication of removed hip and knee prostheses for diagnosis of infection. *N Engl J Med* 2007;**357**:654—63.
32 Puges M, Pereyre S, Bérard X, Accoceberry I, Le Roy C, Stecken L, et al. Comparison of genus specific PCR and culture with or without sonication for microbiological diagnosis of vascular graft infection. *Eur J Vasc Endovasc Surg* 2018;**56**:562—71.
33 Boudjemaa R, Steenkeste K, Jacqueline C, Briandet R, Caillon J, Boutoille D, et al. Live intramacrophagic Staphylococcus aureus as a potential cause of antibiotic therapy failure: observations in an in vivo mouse model of prosthetic vascular material infections. *J Antimicrob Chemother* 2018;**73**:2418—21.
34 Valour F, Trouillet-Assant S, Riffard N, Tasse J, Flammier S, Rasigade JP, et al. Antimicrobial activity against intra-osteoblastic Staphylococcus aureus. *Antimicrob Agents Chemother* 2015;**59**:2029—36.
35 Sjollema J, Sharma PK, Dijkstra RJB, van Dam GM, van der Mei HC, Engelsman AF, et al. The potential for bio-optical imaging of biomaterial-associated infection in vivo. *Biomaterials* 2010;**31**:1984—95.
36 Li D, Gromov K, Søballe K, Puzas JE, O'Keefe RJ, Awad H, et al. Quantitative mouse model of implant-associated osteomyelitis and the kinetics of microbial growth, osteolysis, and humoral immunity. *J Orthop Res* 2008;**26**:96—105.
37 Chauhan A, Lebeaux D, Ghigo JM, Beloin C. Full and broad-spectrum *in vivo* eradication of catheter-associated biofilms using gentamicin-EDTA antibiotic lock therapy. *Antimicrob Agents Chemother* 2012;**56**:6310—8.
38 Van de Vyver H, Bovenkamp PR, Hoerr V, Schwegmann K, Tuchscherr L, Niemann S, et al. A novel mouse model of staphylococcus aureus vascular graft infection: noninvasive imaging of biofilm development in vivo. *Am J Pathol* 2017;**187**:268—79.
39 Garrido V, Collantes M, Barberán M, Peñuelas I, Arbizu J, Amorena B, et al. *In vivo* monitoring of staphylococcus aureus biofilm infections and antimicrobial therapy by [$^{18}$F]fluorodeoxyglucose—microPET in a mouse model. *Antimicrob Agents Chemother* 2014;**58**:6660—7.
40 Coscas R, Senemaud J. Experimenters or amateurs? *Eur J Vasc Endovasc Surg* 2020;**60**:253.