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Presence of a biomaterial implant facilitates induction of experimental infective endocarditis due to streptococci and staphylococci

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Infective endocarditis (IE) usually is studied using animals with catheters inserted into the heart, which causes formation of platelet–fibrin thrombi (vegetations, VGs). We used two rabbit models to study the respective roles of the catheter and the VGs in the development of IE. The influence of the catheter was studied by either removing the catheter before bacterial challenge, or leaving the catheter in place. In all cases, removal of the catheter caused a strong decrease in the frequency of IE. The presence of the catheter stimulated population increase of streptococci within 4 h after challenge. As most catheters were sterile 4 h after challenge, they did not serve as a reservoir of bacteria. To study the requirement of a preformed VG catheters were inserted either 24 h or 30 min before bacterial challenge. In the former model VGs were present, in the latter VGs were not yet formed when bacteria were injected. The frequencies of IE due to 2 *S. sanguis* and 2 *S.epidermidis* strains in the 24 h model or 30 min model were similar, indicating that a preformed VG is not necessary for development of IE. Five coagulase-negative stains were shown to vary in their capacity to cause IE in the 30 min model. Variation was not caused by differences in early adhesion or colonization of the aortic valve, but reflects differences in persistence after initial colonization. Like in the 24 h model, persistence of the bacteria was greatly enhanced by the continuous presence of the catheter. Possible mechanisms of the infection-potentiating effect of the catheters are discussed.

1. Introduction

Infective endocarditis (IE) is an infection of the damaged endothelium of the heart. Cases of endocarditis can be divided into two categories, native and prosthetic valve endocarditis. In native valve endocarditis (NVE) the valve has been damaged by congenital defects or rheumatic fever. Bacteria, in most cases viridans streptococci which enter the circulation from the oral cavity, can adhere to VGs, platelet-fibrin depositions on the damaged heart valves or endothelium. The second category, prosthetic valve endocarditis (PVE), is associated with implantation of a prosthetic valve. PVE infections are further divided into early and late PVE, early PVE being defined as infections arising in the first year after surgery [1]. *Staphylococcus epidermidis* and other coagulase-negative (CN) staphylococci, and *Staphylococcus aureus* are the major causes of early PVE [2, 3].

In experimental animal models endocarditis can be induced by inserting a catheter into the heart [4]. The damage caused gives rise to formation of VGs. After VGs are formed bacteria are injected, which colonize the VGs. In order to cause an infection, bacteria will have to persist and multiply at or in the VGs [4, 5], where they are protected from phagocytic cells. In addition to the VG, the presence of a biomaterial like the catheter may potentiate infection. To study the respective roles of the VGs and the catheter, two models of endocarditis were used, in which catheters were implanted 24 h or only 30 min before injection of bacteria. In the 30 min model no VG was yet formed at the time of bacterial challenge.

2. Materials and methods

2.1. Bacterial strains and growth conditions

Streptococcus sanguis type II strains 1 and 2 [5], as well as *Staphylococcus epidermidis* strains NCTC100835

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(strain 1), NCTC100894 (strain 2), NCTC100892 (strain 3) and SL58 (strain 4), and *Staphylococcus saprophyticus* SAP1 have been used in previous experimental IE studies [5, 6]. Inocula of streptococci and staphylococci grown overnight in Mueller Hinton (MH) or tryptic soy broth (TSB), respectively, were prepared as described [5]. When grown in MH, *S. sanguis* strains 1 and 2 are devoid of dextran [5]. Only *S. epidermidis* strain 4 produced slime, as judged by alcian blue staining of emptied overnight TSB culture tubes [7].

2.2. Two different rabbit models of infective endocarditis

(i) Viridans streptococcal endocarditis model: A polyethylene catheter (Portex, Hythe, Kent, UK) with external and internal diameters of 0.96 mm and 0.58 mm respectively was inserted via the left carotid artery into the left ventricle of New Zealand White rabbits weighing 2.1–3.1 kg, as previously described [6]. To study the influence of the continuing presence of the catheter after bacterial challenge catheters were left in place for 48 h, or catheters were removed 24 h after placement. At 48 h after initial placement of catheters 10^5 *S. sanguis* strain 1 or 2 bacteria in 1 ml PBS were injected into a marginal ear vein. This inoculum corresponded to the 90% infective dose (ID90) for *S. sanguis* strain 1 [5].

(ii) Experimental model for early PVE due to CN staphylococci: Rabbits were catheterized either 24 h ("24 h PVE model") or 30 min ("30 min PVE model") prior to bacterial challenge. This way two markedly different models of early PVE were created. In the 24 h PVE model a VG had already developed at the time of bacterial challenge. In the 30 min PVE model the endocardium and aortic valve (AV) were damaged, but no VG had yet formed. To study the influence of the continuing presence of the catheter after bacterial challenge, in both models the catheter was either left in place or removed just before injection of bacteria. Bacterial inocula consisted of 10^7 CN staphylococci in 1 ml PBS, being the ID90 of *S. epidermidis* strain 2 [7], and were injected into a marginal ear vein.

2.3. Evaluation of bacterial colonization of VGs/AVs and catheters, and of infection

At 5 or 30 min, or 2, 4, or 48 h after bacterial challenge rabbits were sacrificed and bacteria present in homogenized VGs or AVs were quantitatively cultured as described [5]. IE was defined by culture-positivity of VGs at 48 h. Catheter colonization was quantified by culturing segments of catheters in pour plates, as described in [8].

Blood cultures: Blood (5 ml) was drawn from the right ventricle of rabbits immediately after killing and bacteria were quantitatively cultured using pour plates, as described [5].

Statistics: The Wilcoxon two-sample rank sum test was used to calculate the significance of differences between the numbers of CFU recovered from VGs of rabbits with catheters present or removed.

3. Results

Influence of catheter removal on development of viridans streptococcal IE: In the generally applied model of infective endocarditis catheters are inserted into the hearts of test animals 24 or 48 h before bacterial challenge, in order to establish a VG which can readily be colonized by bacteria [4, 6]. We tested whether the continuous presence of the catheter was required for the development of IE. Indeed, in rabbits with catheters removed 24 h before bacterial challenge, the incidence of IE was strongly reduced for *S. sanguis* strains 1 and 2 (Table I). A preformed VG as such, without a catheter present apparently is not sufficient for development of streptococcal IE.

Population dynamics of *S. sanguis* on VGs in presence or absence of the catheter: To investigate the mechanism of the infection-potentiating effect of the catheter, we investigated colonization of the VGs by *S. sanguis* strains 1 and 2 as a function of time. The numbers of CFU 5 min after inoculation show that the initial colonization of VGs by both strains was not influenced by removal of the catheter prior to injection of bacteria (Fig. 1). In the presence of the catheter numbers of *S. sanguis* strain 1 (Fig. 1A) started to increase at 2 h after inoculation, resulting in rapid

TABLE I Culture positive VGs 5 min and 48 h after inoculation of rabbits with 10^5 *Streptococcus sanguis* or 10^7 *Staphylococcus epidermidis*, in the 24 h model[a] of IE.

Strain[b]	Catheter left in place[a]		Catheter removed[a]	
	Culture-positive/total VGs at 5 min	after 48 h	Culture-positive/total VGs at 5 min	after 48 h
S. sanguis				
strain 1	30/35	15/16	6/6	0/8
strain 2	10/12	12/13	ND	3/10
S. epidermidis				
strain 1	12/12	4/11	12/12	0/9
strain 2	12/12	8/10	12/12	2/9

[a] In the experiments with *S. sanguis*, catheters were inserted into the left heart of rabbits, and were either left in place or removed after 24 h. After an additional 24 h bacteria were injected. In the experiments with *S. epidermidis* catheters were inserted and after 24 h either left in place or removed prior to bacterial challenge.

[b] Inocula of 10^5 or 10^7 CFU of streptococci and staphylococci, respectively, were injected into a marginal ear vein.

ND: not done.

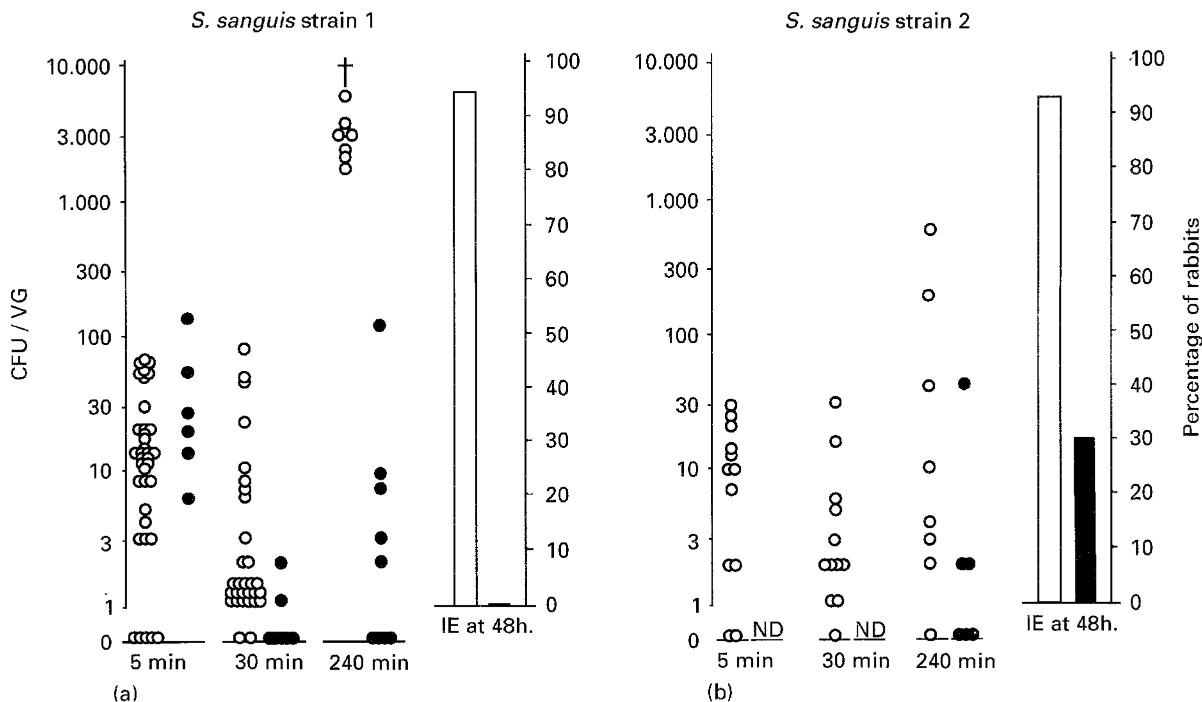

Figure 1 Numbers of CFU per VG and development of IE in rabbits with catheters present or removed before injection of 10^5 streptococci. Catheters were inserted into the left heart of rabbits, and were either left in place (white circles) or removed after 24 h (black circles). After an additional 24 h bacteria were injected. A dagger indicates p < 0.01 versus the values obtained at the corresponding time point in rabbits from which catheters were removed. ND means not done.

expansion of the population at 4 h. When the catheter was removed, no such increase was observed with time, and bacteria disappeared from the VGs. *S. sanguis* strain 2 showed similar dynamics (Fig. 1B), but expansion of the population in the presence of the catheter was slower than for *S. sanguis* strain 1.

Influence of catheter removal on development of IE due to *S. epidermidis* in the 24 h PVE model: Catheters inserted into the hearts of rabbits were either left in place or removed after 24 h, prior to challenge with *S. epidermidis* strains 1 or 2. Initial colonization of VGs was not influenced by removal of catheters, as all VGs were culture positive at 5 min after challenge. Final development of IE after 48 h was reduced in rabbits from which catheters had been removed (Table I).

Population dynamics of *S. epidermidis* in the presence or absence of a catheter in the 24 h PVE model: Although development of IE after 48 h due to *S. epidermidis* strains 1 and 2 was clearly enhanced by prolonged catheter presence, expansion of the populations on the VGs as observed with *S. sanguis* strain 1 had not yet started at 4 h after challenge (Table I, Fig. 2).

Role of catheter–colonization in catheter–enhanced bacterial population increase on VGs: Catheter colonization was studied in the 24 h model with *S. sanguis* strains 1 and 2, and *S. epidermidis* strains 1 and 2. *S. sanguis* strain 1, which showed rapid population increase at 4 h (Fig. 1A), did not show a high initial colonization of the catheter 5 min after injection. Four hours after inoculation catheters were sterile (Fig. 3). The data for *S. sanguis* strain 2 were similar, although at 4 h 12% of the catheters still contained a low number of bacteria. The two *S. epidermidis* strains adhered to catheters more avidly at 5 min after inoculation, but were removed from most of the catheters at 4 h. Apparently the catheters did not function as a reservoir of bacteria. This is in accordance with the fact that blood cultures always were negative at 4 h.

Frequencies of IE due to staphylococci in the 24 h PVE model and the 30 min PVE model: Frequencies of IE due to *S. epidermidis* strains 1 and 2 in the 30 min model with catheters left in place were similar to the frequencies in the 24 h model with catheters left in place (strain 1: 9/23 (30 min model) and 4/11 (24 h model); strain 2: 21/25 (30 min model) and 8/10 (24 h model), (Tables I and II). In the presence of a catheter, a preformed VG apparently is not a prerequisite for development of IE.

The frequencies of IE after 48 h in the presence of the catheter due to the 5 staphylococcal test strains in the 30 min PVE model showed considerable variation. *S. saprophyticus* SAP1 did not cause IE, and *S. epidermidis* strains 2 and 3 were more virulent than strains 1 and 4. Remarkably, 5 min after injection almost all AVs were culture positive for all strains (Table II). The strains thus have different abilities to persist in the presence of the catheter.

Influence of catheter removal on bacterial colonization of VGs in the 30 min PVE model: We tested whether prolonged presence of the catheter was required for development of IE in the 30 min PVE model. As in the 24 h PVE model, the frequencies of IE after 48 h in the 30 min PVE model were dramatically reduced when catheters were removed prior to inoculation (Table II). Strains 2 and 3, which caused the highest frequencies of IE when catheters were left in place, caused IE in 40% and 20% of rabbits from which catheters had been removed.

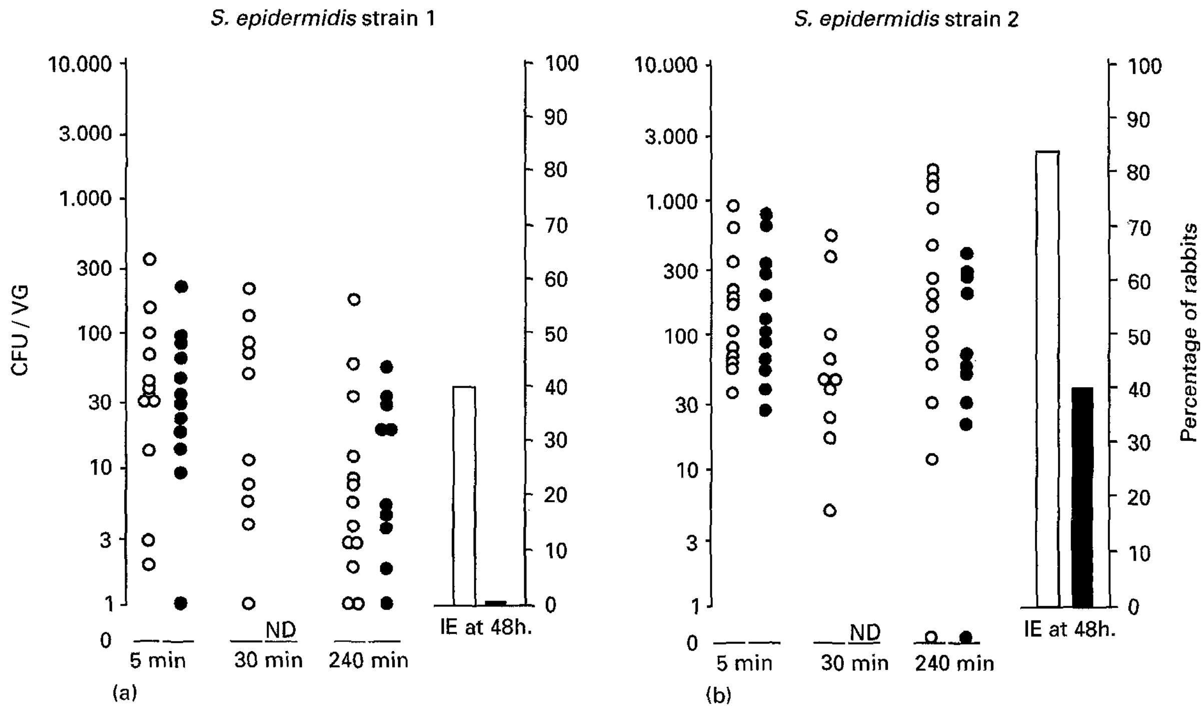

Figure 2 Numbers of CFU per VG and development of IE in rabbits with catheters present or removed before injection of 10^7 staphylococci. Catheters were inserted and after 24 h either left in place (white circles) or removed (black circles) prior to bacterial challenge. ND means not done.

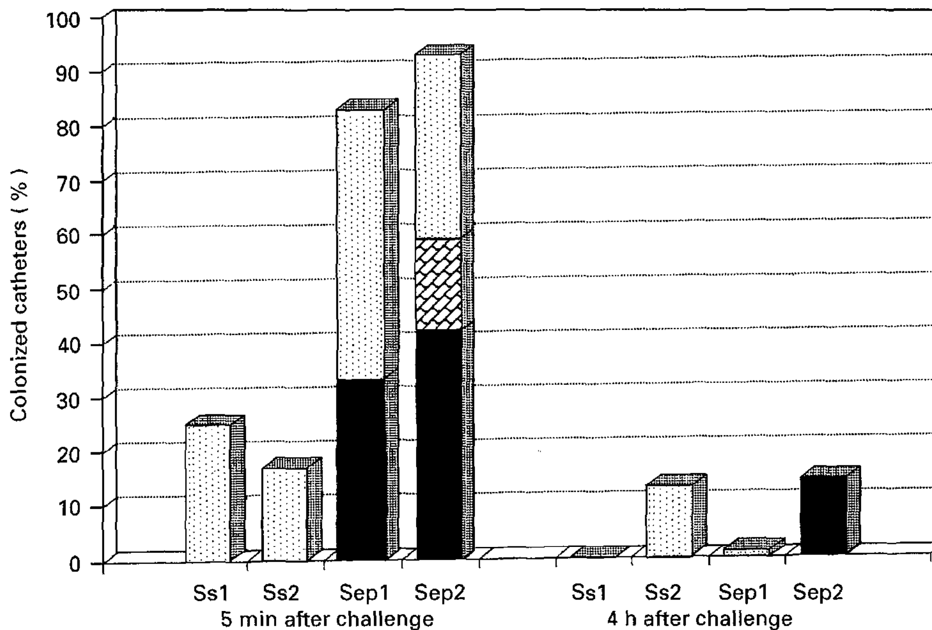

Figure 3 Percentage of culture-positive catheters at 5 min and 4 h after challenge of rabbits with 10^5 *S. sanguis* strain 1 (Ss1) or strain 2 (Ss2), or 10^7 *S. epidermidis* strain 1 (Sep1) or strain 2 (Sep2). Catheters were inserted into the left heart of rabbits 48 or 24 h before challenge with streptococci or staphylococci, respectively, and left in place. (▤), 1–5; (▨), 5–10; (■) > 10 CFU.

4. Discussion

Infective endocarditis generally is studied using animal models with catheters inserted into the heart. Insertion of the catheter is performed 24–72 h before injection of the bacteria. This causes formation of a sterile thrombotic VG, which is considered to be necessary for development of IE. This type of model mimics the situation of the natural valve endocarditis or late PVE. The trauma caused by the catheter can be considered to reflect the local traumata on valves and endocardium as caused by abberant blood flow due to congenital or acquired defects in the heart. However, in addition to the VG, the catheter itself may contribute to the development of IE.

If the VG as such would be sufficient for development of IE, removal of the catheter 24 h after insertion, but before injection of bacteria, would not have any effect on the development of IE. However, removal of the catheter before bacterial challenge dramatically reduced the development of IE due to two *S. sanguis* strains and two *S. epidermidis* strains (Table I). This difference is not caused by differences

TABLE II Culture positive AVs at 5 min and VGs at 48 h after inoculation of rabbits with CN staphylococci in the 30 min PVE model[a] of IE

Strain[b]	Catheter left in place[a]		Catheter removed[a]
	Culture-positive/total AVs or VGs		Culture-positive/total VGs
	at 5 min	after 48 h	after 48 h
S. epidermidis			
strain 1 (100835)	19/19	9/23	0/8
strain 2 (100894)	19/19	21/25	4/10
strain 3 (100892)	13/15	7/10	2/10
strain 4 (SL58)	12/12	3/10	0/10
S. saprophyticus			
SAP1	9/9	0/11	0/8

[a] Catheters were inserted into the left heart of rabbits 30 min before bacterial challenge, and were either left in place or removed directly prior to bacterial challenge.
[b] Inocula of 10^7 CFU of staphylococci were injected into a marginal ear vein.

in initial colonization in the presence or absence of catheters (Fig. 1), but is related to the fact that only in the presence of the catheter bacterial populations increase with time (Figs 1 and 2). The presence of the VG as such thus is not sufficient for development of IE.

In most cases of early prosthetic valve endocarditis infections are due to contamination with bacteria, commonly CN staphylococci, which occurs during implantation [9, 10] or very shortly thereafter [11]. In order to simulate this situation, we designed a new model to study experimental early prosthetic valve endocarditis. In this model the catheter is inserted into the heart of rabbits only 30 min before injection of bacteria. At the time of inoculation a VG has not yet formed. When the catheters remained in place after bacterial challenge, the frequencies of IE due to 2 *S. epidermidis* strains in this 30 min PVE model were similar to those observed in the 24 h model (Tables I and II). Apparently, the presence of a preformed, macroscopic VG is not required for development of IE. Like in the 24 h PVE model, in the 30 min PVE model the presence of the catheter strongly enhanced development/onset of IE. Despite the absence of a preformed VG bacteria in the circulation may adhere to the freshly traumatized tissue where microthrombi are forming. They will then be embedded in the depositing platelets and fibrin of the growing VG, and will be protected from phagocytes. Since the 30 min model more realistically mimics the situation of early PVE than existing models of IE, this model can contribute to more accurate studies on the pathogenic mechanisms underlying early PVE due to CN staphylococci.

Our data seem to contradict those obtained in a rat model of endocarditis, in which catheters were inserted three days before bacterial challenge, and were either left in place or removed 30 min before injection of bacteria [12]. All animals challenged with 10^7 *Staphylococcus aureus* or *Streptococcus intermedius* developed IE, regardless of the presence of the catheter. In the presence of the catheter however, higher population densities were found. The discrepancy between these observations and our finding that the catheter enhances infection due to *S. sanguis* and *S. epidermidis* may be due to the fact that in rats the inoculum size of 10^7 staphylococci or streptococci was too large to detect the infection-potentiating effect of the catheter [12]. As in our studies, an infection-potentiating effect of the catheter was noted when a serum-resistant *Escherichia coli* strain was used in rats [12, 13]. The moment of catheter removal relative to the moment of injection of bacteria was shown to be of crucial importance for the development of IE due to *E. coli*. The frequency of IE ranged from 33% when the catheter was removed 1 h before challenge, to 91% when removal took place 6 h after bacterial challenge [13]. Apparently the first hours after injection of bacteria are decisive for development of IE, which is strongly enhanced by the presence of a catheter.

The infection-potentiating effect of the catheters can be compared to other situations in which implanted biomaterials have been shown to enhance infections [14–16]. We have shown that the catheter does not function as a reservoir of bacteria (Fig. 3). In the presence of the catheter however, bacterial populations start to increase 4 h after injection or shortly thereafter (Figs 1 and 2). This might be explained by either (i) reduced effectivity of local host defences, or (ii) enhanced growth of the bacteria, or a combination of both factors.

The most obvious element involved in rapid local host defence is phagocytosis by polymorphonuclear phagocytes (PMNs). Indeed PMNs isolated from tissue cages have been shown to be impaired in their phagocytic and bactericidal properties [17, 18]. However, these PMNs were isolated from the tissue cages 14 days after implantation, when the foreign body inflammatory response had been stabilized. Injection of *Staphylococcus aureus* bacteria into such tissue cages caused recruitment of fresh PMNs with full phagocytic/bactericidal capacity [17]. Furthermore, the role of PMNs in IE is considered to be limited by the fact that bacteria are shielded from the phagocytes by the fine fibrin/platelet meshwork of the VG.

A second explanation for the infection-potentiating effect of biomaterials could be the enhancement of growth of the bacteria. In the case of experimental IE the catheter causes continuous (sub)endothelial damage, which induces an inflammatory response. Due to

the direct cell damage as well as the activation of inflammatory cells and endothelium, numerous potentially nutritious compounds will continuously be exuded in the vicinity of the catheter. As the wound environment [16] as well as several specific inflammatory mediators [19–21] have been shown to enhance bacterial growth, the inflammatory environment may be beneficial for bacterial population expansion. Thus, the presence of the biomaterial may shift the balance of bacterial population decrease by the host defence versus population increase by enhanced growth to the benefit of the bacteria, thereby potentiating for infection.

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