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Historical perspective

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ABSTRACT

Bacterial adhesion is a main problem in many biomedical, domestic, natural and industrial environments and forms the onset of the formation of a biofilm, in which adhering bacteria grow into a multi-layered film while embedding themselves in a matrix of extracellular polymeric substances. It is usually assumed that bacterial adhesion occurs from air or by convective-diffusion from a liquid suspension, but often bacteria adhere by transmission from a bacterially contaminated donor to a receiver surface. Therewith bacterial transmission is mechanistically different from adhesion, as it involves bacterial detachment from a donor surface followed by adhesion to a receiver one. Transmission is further complicated when the donor surface is not covered with a single layer of adhering bacteria but with a multi-layered biofilm, in which case bacteria can be transmitted either by interfacial failure at the biofilm-donor surface or through cohesive failure in the biofilm. Transmission through cohesive failure in a biofilm is more common than interfacial failure. The aim of this review is to oppose surface thermodynamics and adhesion force analyses, as can both be applied towards bacterial adhesion, with their appropriate extensions towards transmission. Opposition of surface thermodynamics and adhesion force analyses, will allow to distinguish between transmission of bacteria from a donor covered with a (sub)monolayer of adhering bacteria or a multi-layered biofilm. Contact angle measurements required for surface thermodynamic analyses of transmission are of an entirely different nature than analyses of adhesion forces, usually measured through atomic force microscopy. Nevertheless, transmission probabilities based on Weibull analyses of adhesion forces between bacteria and donor and receiver surfaces, correspond with the surface thermodynamic preferences of bacteria for either the donor or receiver surface. Surfaces with low adhesion forces such as polymer-brush coated or nanostructured surfaces are thus preferable for use as non-adhesive receiver surfaces, but at the same time should be avoided for use as a donor surface. Since bacterial transmission occurs under a contact pressure between two surfaces, followed by their separation under tensile or shear pressure and ultimately detachment, this will affect biofilm structure. During the compression phase of transmission, biofilms are compacted into a more dense film. After transmission, and depending on the ability of the bacterial strain involved to produce extracellular polymeric substances, biofilm left-behind on a donor or transmitted to a receiver surface will relax to its original, pre-transmission structure owing to the viscoelasticity of the extracellular polymeric substances matrix, when present. Apart from mechanistic differences between bacterial adhesion and transmission, the low numbers of bacteria generally transmitted require careful selection of suitably sensitive enumeration methods, for which culturing and optical coherence tomography are suggested. Opposing adhesion and transmission as done in this review, not only yields a better understanding of bacterial transmission, but may stimulate researchers to more carefully consider whether an adhesion or transmission model is most appropriate in the specific area of application aimed for, rather than routinely relying on adhesion models.

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Contents

1. Introduction

The biofilm mode of growth is greatly preferred by most bacterial strains and species [1,2]. The sequence of events leading to biofilm formation is generally considered to commence with bacterial transport by convective-diffusion (Fig. 1A) from a liquid suspension to a substratum surface or impingement from aerosols (Fig. 1B). Initially, bacterial adhesion is reversible, but production of EPS can rapidly lead to an irreversible state and subsequent growth of a bacterial (sub)monolayer into a multi-layered biofilm (Fig. 1C). Bacterial transmission is a less commonly highlighted means of bacterial transport, but equally if not more prevalent than adhesion in many biomedical, domestic, natural and industrial environments. Although many parameters are influential upon bacterial transmission, including temperature, humidity, type of contact pressure (shear or compression) and duration, physico-chemically it is important to distinguish between transmission from a donor surface contaminated with a (sub)monolayer of adhering bacteria (Fig. 2A) or from a donor fully covered with a multi-layered bacterial biofilm (Fig. 2B). In the latter case, transmission from a donor surface can occur either through cohesive failure in the biofilm or interfacial failure at the donor-biofilm interface (see also Fig. 2B). Mechanistically different from bacterial adhesion, transmission involves detachment from a donor surface and adhesion to a receiver surface [3,4].

The aim of this review is to oppose surface thermodynamics and adhesion force analyses, as can both be applied towards bacterial adhesion, with their appropriate extensions towards transmission and reveal their respective merits in explaining bacterial transmission phenomena. Furthermore, the impact of the viscoelastic EPS matrix on biofilm structure after transmission is discussed. Since often low numbers of bacteria are involved in transmission, advantages and disadvantages of different methods to study bacterial transmission are compared and methods considered appropriate are highlighted.

2. The importance of bacterial transmission

Before embarking on the physico-chemical differences between bacterial adhesion and transmission, the general importance of transmission in different environments will first be briefly highlighted.

2.1. Bacterial transmission in biomedical environments

Bacterial transmission frequently occurs in hospital environments and nursing homes among hands of healthcare workers [5] and patients [6], including biomaterial implants or devices and environmental surfaces in hospitals. Bacterial transmission between patients with an indwelling urinary catheter for instance, was three times higher when nursed in the same room than when nursed in separate rooms [7], while patients admitted to rooms previously occupied by patients with methicillin resistant *Staphylococcus aureus* (MRSA), vancomycin resistant *Enterococcus* (VRE) or *Acinetobacter baumannii* had a 73% increased risk of acquiring the same pathogen from environmental surfaces [8]. The World Health Organization reported that on average 8.7% of hospitalized patients acquired nosocomial infections due to bacterial transmission [9], which is a particular risk for immunocompromised patients [10] or patients with biomaterial implants or devices [11]. Bacterial transmission also occurs during insertion of indwelling urinary [12] or vascular catheters [13], either from the peri-urethral area or subcutaneous layers of the skin [14], respectively. Similarly, endoscopes become contaminated with bacteria during use [15], bacteria become transmitted to other surfaces in radiography machines [16], computer equipment acts as fomites for bacterial transmission equally as gloves [17], light handles [18] and surgical appliances in the operating theater [19]. Transmission of microorganisms from contaminated lens cases to contact lenses followed by transmission to the cornea is a well-known cause of microbial keratitis, posing a general

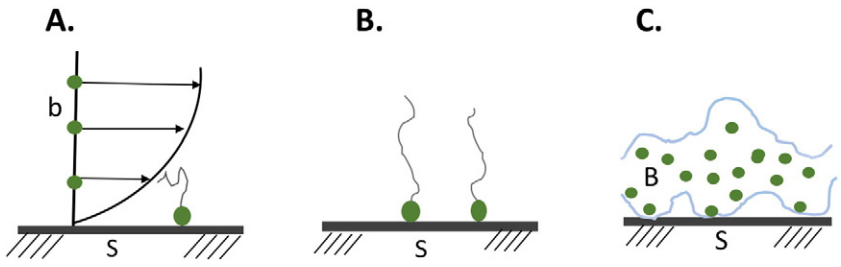

Fig. 1. Transport in bacterial adhesion to a substratum surface (S) and biofilm growth. A. Bacterial (b) transport from a flowing suspension by convective-diffusion. B. Bacterial transport by impingement from aerosols. C. A multi-layered biofilm (B) resulting from growth of adhering bacteria.

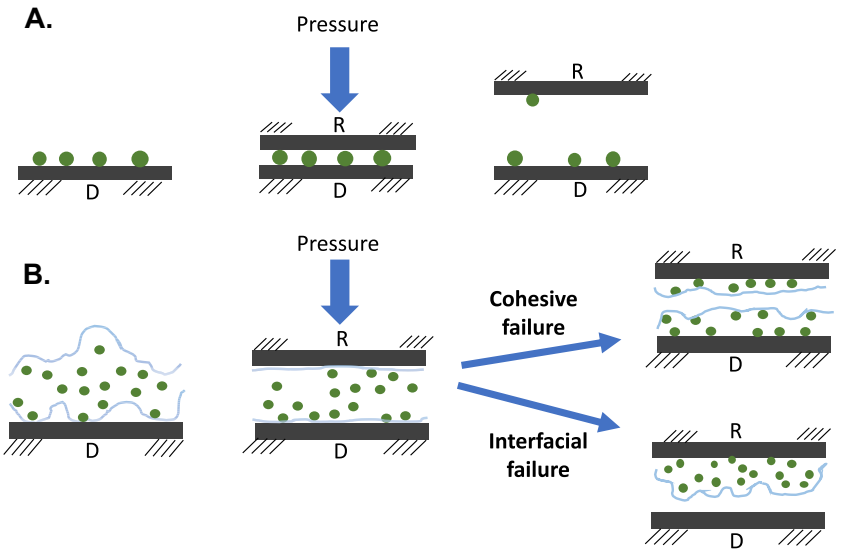

Fig. 2. Distinction between bacterial transmission from a (sub)monolayer of contaminating bacteria versus transmission from a multi-layered biofilm. A. Transmission from a bacterial (sub)monolayer to a receiver (R) surface, involving interfacial failure at the donor (D)-bacterium interface. B. Transmission of bacteria from a multi-layered biofilm to a receiver surface, involving either cohesive failure in the biofilm or interfacial failure at the donor-biofilm interface.

healthcare threat due to the large number of people wearing contact lenses [20]. Toothbrushes are mentioned more and more as a source for microbial transmission [21].

2.2. Bacterial transmission in domestic environments

Bacterial transmission in domestic environments is inevitable, but usually involves less pathogenic microorganisms than present in biomedical environments [22]. Bacterially contaminated fabrics have the potential to contaminate laundry in washing machines [23], as well as washing machines themselves, which can lead to contamination of subsequent loads of laundry [23,24]. Microorganisms in fresh, unprepared food can transmit to kitchen surfaces [25] and onto household members through handling devices during preparation [22]. Telephone receivers are an intermediate for transmission of bacteria from one user to the next [26]. Money is frequently contaminated by pathogens from the intestinal and respiratory tract [27] and adhering bacteria on bank notes and coins can be transmitted from hand to hand, sometimes ongoing to food [28].

2.3. Bacterial transmission in natural and industrial environments

Bacterial transmission also occurs in natural and industrial environments, including slaughterhouses, agricultural, forest and sea-water environments. Particularly in food processing, bacterial transmission can lead to a rapid spread of potential pathogens. In slaughter houses, *Campylobacter* has been found colonizing employees boots and clothes [29], which may lead to bacterial transmission to carcasses (and vice versa) [30]. Knives are subsequently notorious sources of bacterial transmission from contaminated meat to uncompromised meat of other animals, that may proceed to household kitchen appliances and consumers [31]. In forest areas, bacteria shed by wild animals can persist in seeds, water, manure or feed, and spread to agriculture areas and contaminate farmers and their livestock [32]. Also, contaminated seeds can transmit bacteria to non-contaminated plants [33]. In seawater, bacteria shed by infected fish can survive for some time and transmit to other susceptible fish [34] onto the food chain.

3. Mechanism of bacterial transmission

Bacterial transmission depends critically on the relative affinity of adhering bacteria for the donor and receiver surfaces, or for other biofilm inhabitants. Conceptually, bacterial affinity can be specified in many ways [35]. In order to oppose bacterial adhesion and transmission, we will here describe bacterial transmission in terms of common physico-chemical mechanisms described for bacterial adhesion to surfaces [36], i.e. a surface thermodynamic approach and an analysis based on adhesion forces.

3.1. Surface thermodynamics of bacterial transmission

In a surface thermodynamic approach, bacterial adhesion to surfaces is considered favorable when the interfacial free energy of adhesion $\Delta G_{adh} < 0$. ΔG_{adh} can be calculated from the interfacial free energies γ_{bl}, γ_{sl} and γ_{sb}, as outlined in Fig. 3A [37]. The interfacial free energies can be calculated from measured contact angles θ with liquids possessing different polarities on substratum surfaces and macroscopic lawns of organisms prepared on membrane filters [38,39], while the polarities of different liquid and their surface tension can be taken from the literature [40]. There are various ways to calculate the interfacial free energies from measured contact angles with liquids that we consider outside the scope of this review to compare [39]. One of the most common approaches however, is the Lifshitz-Van der Waals/acid-base approach [41]:

$$\cos\theta = -1 + \frac{2\sqrt{\gamma_{sv}^{LW}\gamma_{lv}^{LW}}}{\gamma_{lv}} + \frac{2\sqrt{\gamma_{sv}^{+}\gamma_{lv}^{-}}}{\gamma_{lv}} + \frac{2\sqrt{\gamma_{sv}^{-}\gamma_{lv}^{+}}}{\gamma_{lv}} \quad (1)$$

in which γ_{sv}^{LW}, γ_{bv}^{LW}, and γ_{lv}^{LW} denote the Lifshitz–Van der Waals component of the surface free energy of the substratum surface, the bacterial cell surface or the liquid phase, respectively. γ_{lv} is the surface free energy of the liquid–vapor interface. The acid–base components of the surface free energies are accordingly indicated as γ^{AB} and can be separated into an electron-donating (γ^{-}) and electron-accepting (γ^{+}) parameter according to

$$\gamma^{AB} = 2\sqrt{\gamma^{-}\gamma^{+}} \quad (2)$$

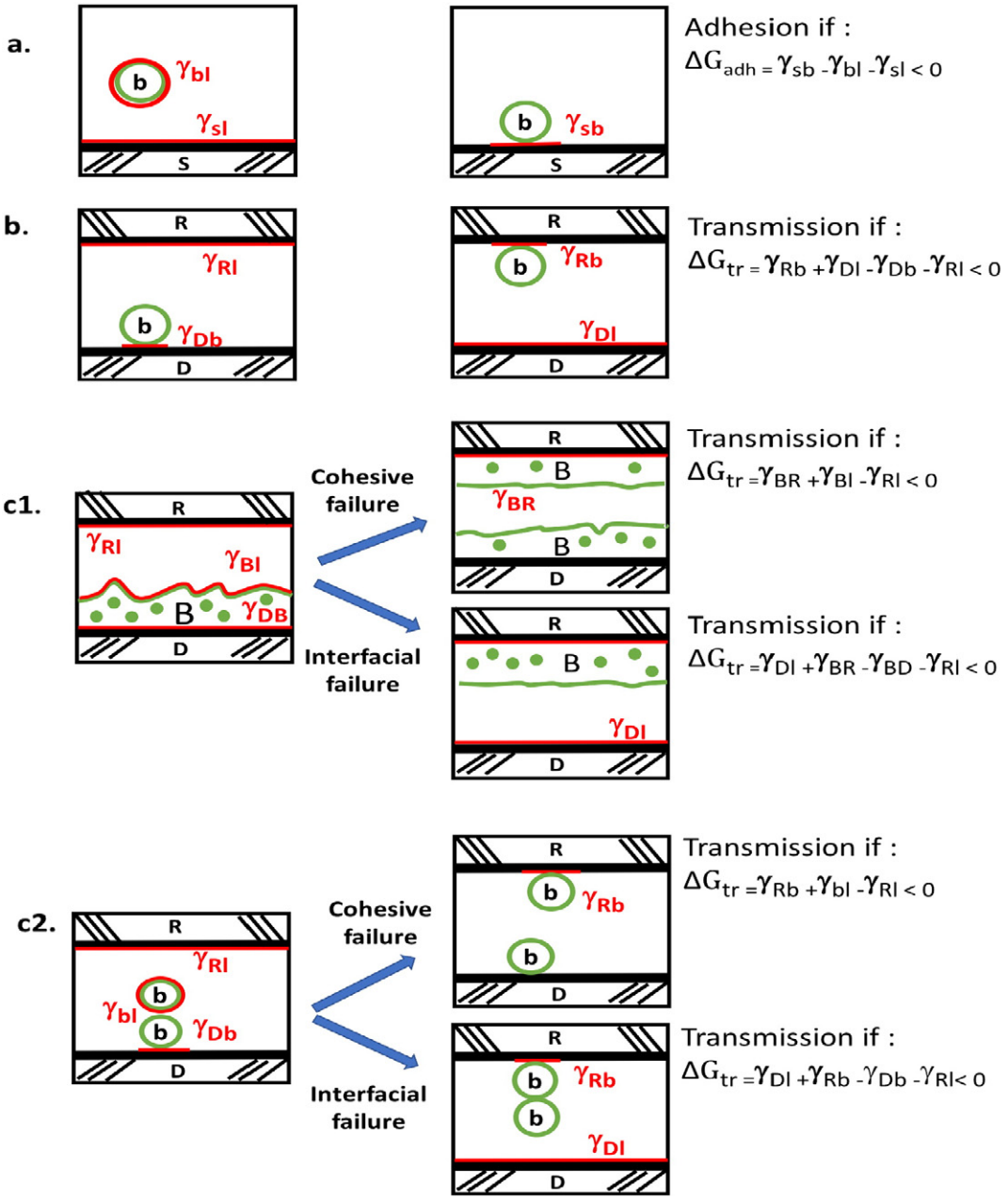

Fig. 3. Surface thermodynamics of bacterial adhesion versus transmission from a (sub)monolayer of contaminating bacteria or from a multi-layered biofilm. A. Comparison of interfacial free energies for a bacterium (b) in an aqueous suspension (l) and adhering to a substratum surface (s), yielding the interfacial free energy of adhesion ΔG_{adh}. B. Comparison of interfacial free energies for bacteria adhering in (sub)monolayer on a donor and receiver surface (D and R, respectively), yielding the interfacial free energy of transmission ΔG_{tr}. C1. Comparison of interfacial free energies for bacteria adhering in a multi-layered biofilm (B) on a donor and receiver surface, while embedded in an EPS matrix without direct contact between bacteria for cases of interfacial and cohesive failure, yielding the interfacial free energy of transmission ΔG_{tr}. C2. Comparison of interfacial free energies for bacteria adhering in a multi-layered biofilm B on a donor and receiver surface with direct contact between bacteria for cases of interfacial and cohesive failure, yielding the interfacial free energy of transmission ΔG_{tr}.

Since Eq. (1) contains three unknowns when a new surface is to be analyzed for its surface free energy parameters and components, it requires contact angle measurements with three distinctly different liquids to solve Eq. (1) for γ^{LW}, γ^- and γ^+. Drawback of the use of surface thermodynamics to bacterial adhesion is that very often bacterial adhesion does not meet the thermodynamic requirement of being reversible, because bacterial adhesion becomes already irreversible after several seconds to minutes [42]. Furthermore, not seldom the interface between a bacterium and a substratum surface is highly dynamic over time, as bacterial surface appendages collapse during adhesion to a surface and the gradual bond-maturation to an irreversible state [42–44]. Nevertheless, cases in which $\Delta G_{adh} < 0$, have been found to be associated with less reversible adhesion than when $\Delta G_{adh} > 0$ [45–47].

The concept of interfacial free energy of adhesion can be readily applied to derive an interfacial free energy of transmission to determine whether transmission from contaminating bacterial (sub)monolayers (see Fig. 3B) is thermodynamically favorable ($\Delta G_{tr} < 0$), according to [48]

$$\Delta G_{tr} = (\Delta G_{adh})_{receiver} - (\Delta G_{adh})_{donor} \quad (3)$$

in which ΔG_{tr} is the interfacial free energy of transmission between a donor and receiver surface for which the interfacial free energies of adhesion equal $(\Delta G_{adh})_{donor}$ and $(\Delta G_{adh})_{receiver}$, respectively. For transmission of bacteria adhering in multi-layered biofilms (compare Fig. 3B and C), equations are more complex than in case of transmission from a

(sub)monolayer of contaminating bacteria. In a multi-layered biofilm, bacteria are embedded in an EPS matrix that prevents direct contact between bacteria. Moreover, although entire biofilms might theoretically be transmitted from a donor to a receiver surface, most studies have shown that donor surfaces remain fully covered with biofilm after transmission, while the receiver surface can become either partly or fully covered by transmitted biofilm as well [49,50]. This has yielded the conclusion that bacterial transmission from a biofilm occurs mainly through cohesive failure in the biofilm and not through interfacial failure at the donor-biofilm interface (Fig. 3 C1). Thermodynamically, whether or not cohesive or interfacial transmission occurs, depends on the relative magnitudes of the interfacial free energies of transmission for both situations, depicted in Fig. 3 C1.

Whereas in naturally grown biofilms the distance between bacteria has been estimated to range between 1 and 3 μm, far beyond the reach of physico-chemical interaction forces [51], contact pressures are exerted during transmission that increase the volumetric density of bacteria in a biofilm and therewith decrease the distances between inhabiting bacteria. This compression may yield the scenario depicted in Fig. 3 C2 in which biofilm inhabitants are actually in direct contact with each other, although this yields essentially similar equations for the interfacial free energy of transmission as the scenario in which bacteria are transmitted from an uncompressed biofilm. Nevertheless, there are major differences between the surface free energy of single bacteria, deposited in a bacterial lawn [46,48] as occurring in the equations governing transmission from contaminating (sub)monolayers and the surface free energy of a biofilm of the same strain [52].

The implications of these thermodynamic considerations are summarized in Table 1B for transmission from bacterial (sub)monolayers, using input data of hypothetical substrata and bacteria used, as summarized in Table 1A. Hydrophobic and hydrophilic bacteria have been given properties roughly representative for both types of physico-chemically different types, based on a reference guide of 142 different bacterial strains [53].

Table 1B firstly shows that bacterial adhesion between identical donor and receiver surfaces is not accompanied by any thermodynamic preference. Hydrophobic bacteria do not like to be transmitted from hydrophobic surfaces to hydrophilic ones, but oppositely are eager to transmit from a hydrophilic donor to a hydrophobic receiver. The hypothetical, hydrophilic bacterium basically shows the same trends as the hydrophobic bacterium but with less extreme thermodynamic preferences. The appearance of positive values of the interfacial free energy of the hydrophilic bacterium on a hydrophilic surface may at first seem puzzling, but indicates that water has a bigger preference for that surface than the hydrophilic organisms. In case a more hydrophilic bacterium would have been chosen, results would have been different. Moreover, it should be noted that bacteria usually adhere also in case of unfavorable thermodynamic conditions as a result of the dynamic behavior of bacterial cell surface components that may differ in different environments, e.g. during contact angles measurements and when interfacing a substratum surface [54]. However, detachment tendencies of adhering bacteria have been demonstrated to be in accordance with predictions based on interfacial free energies of adhesion [47,55,56]

Table 1A
Surface free energy components and parameters of the aqueous phase and hypothetical[a] hydrophobic and hydrophilic substrata and bacteria used to illustrate the implication of surface thermodynamics for bacterial transmission among these substrata.

Substance	γ^{LW} (mJ m^{-2})	γ^{-} (mJ m^{-2})	γ^{+} (mJ m^{-2})
Aqueous phase	22	25	25
Hydrophobic substratum	20	2	0
Hydrophilic substratum	30	50	4
Hydrophobic bacterium	30	4	4
Hydrophilic bacterium	40	40	2

[a] Properties of hypothetical hydrophobic and hydrophilic bacteria are based on a published reference guide on bacterial surface free energies [53].

Table 1B
Illustration of the implications of surface thermodynamics for bacterial transmission from bacterial (sub)monolayers among hydrophobic and hydrophilic donor and receiver surfaces for a hydrophobic and hydrophilic bacterial strain in an aqueous phase (for input data see Table 1A).

Donor	Receiver	$\Delta G_{adh,\ DONOR}$ (mJ m^{-2})	$\Delta G_{adh,\ RECEIVER}$ (mJ m^{-2})	ΔG_{tr} (mJ m^{-2})
Hydrophobic bacterium				
Hydrophobic	Hydrophobic	−53	−53	0
Hydrophobic	Hydrophilic	−53	−8	+45
Hydrophilic	Hydrophobic	−8	−53	−61
Hydrophilic	Hydrophilic	−8	−8	0
Hydrophilic bacterium				
Hydrophobic	Hydrophobic	−13	−13	0
Hydrophobic	Hydrophilic	−13	20	+33
Hydrophilic	Hydrophobic	20	−13	−33
Hydrophilic	Hydrophilic	20	20	0

and the same will be true for bacterial detachment from the donor during bacterial transmission. The exact role of surface thermodynamics in bacterial adhesion to a receiver surface during transmission [42,44] is relatively uncertain. Oppositely, the role of interfacial free energies of adhesion in bacterial detachment from a donor surface during transmission [47,55,57] is more established. This is in line with a previous conclusion that donor surface free energies are more influential on bacterial transmission than receiver ones, as bacteria have to detach from the donor and adhere to the receiver surface [48]. Also hydrophobic *Listeria monocytogenes* adhered more strongly to hydrophobic surfaces than hydrophilic ones, leading to less transmission [58]. Indeed, favorable thermodynamic conditions for bacterial transmission ($\Delta G_{tr} < 0$) have been shown to be accompanied by higher transmission probabilities, also as calculated from force analyses using atomic force microscopy (AFM) [48] (see Section 3.2 and Fig. 4).

3.2. Adhesion force analysis of bacterial transmission

In an adhesion force analysis, bacterial transmission between surfaces is considered favorable when the adhesion force of the bacteria to the receiver surface is larger than the adhesion force to the donor surface. Bacterial adhesion forces to substratum surfaces [59,60] but also between two different bacteria [61,62] or a bacterium and an existing biofilm [63], can be measured using single bacterial probe AFM [64,65]. However, AFM has shown that for many bacterial strains and

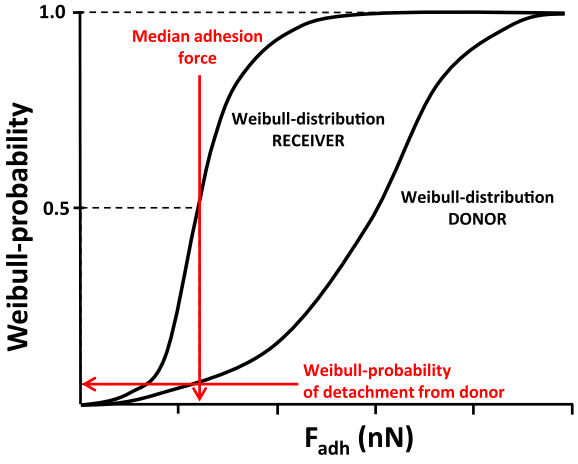

Fig. 4. Weibull-probabilities of the occurrence of bacterial adhesion force values on a donor and receiver surface as a function of the adhesion force. Bacterial transmission probability is taken as the Weibull-probability that the median force by which the bacteria adhere to the receiver is able to detach a bacterium from the donor surface, according to the Weibull-distribution for the donor.

species, whole cell adhesion forces to different negatively-charged substratum surfaces group rather closely together [66,67], with some studies indicating that in general, bacterial strains may adhere more strongly to hydrophobic surfaces [68–70]. Bacterial adhesion forces to polymer-brush coated substratum surfaces have been described throughout the literature as being lowest [71,72], while extremely strong adhesion forces were measured on positively-charged surfaces [73,74]. With the exception of extreme values as on polymer-brush coatings and positively-charged surfaces, the wide variations observed in bacterial adhesion forces often makes statistically significant comparisons of adhesion forces on donor and receiver surfaces difficult.

However, large variations not only occur in microscopic fracture analysis, which is in essence what bacterial adhesion force measurements in AFM represent, but also in macroscopic failure analysis of larger structures [75]. Weibull analysis takes advantage of these large standard deviations to calculate a failure probability [76] and can also be applied to bacterial adhesion forces [48]. As a first step in Weibull analysis, all adhesion forces N in a given data set are ranked in ascending order to calculate the probability P_F of a force value F to occur according to

$$P_F = \frac{n}{N+1} \tag{4}$$

in which n is the rank number. Then, P_F is fitted to the Weibull-equation

$$P_F = 1 - \exp\left\{-\frac{F - F_u}{F_n}\right\}^m \tag{5}$$

in which constant F_u is the lowest level of force at which P_F approaches zero. The constant F_n is generally referred to as a normalizing parameter. The constant m is the dependability of the bond ("Weibull-modulus") [48,56].

Comparison of the Weibull-distribution of bacterial adhesion forces observed in AFM for donor and receiver surfaces, can next be used to calculate a transmission probability (see also Fig. 4). This transmission probability is taken as the probability that an adhering bacterium will detach from a donor surface by a force, similar to the median adhesion force exerted by the receiver surface.

Interestingly, trends in bacterial transmission probabilities calculated from Weibull-distributions of bacterial adhesion forces on donor and receiver surfaces coincided with predictions based on surface thermodynamic analyses of the donor, receiver and bacterial cell surface free energies involved (see Fig. 5), although the linear correlation R^2 (0.53) was low [48].

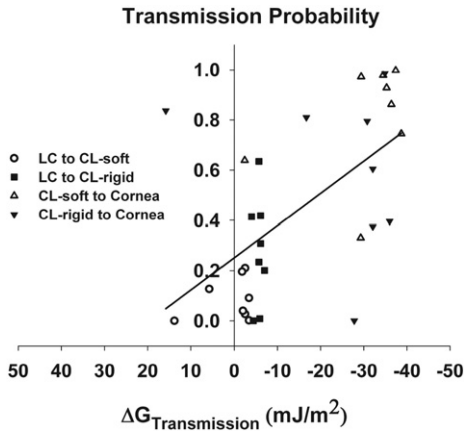

Fig. 5. Bacterial transmission probabilities according to a comparison of the Weibull distributions for bacterial adhesion forces as a function of the interfacial free energies of transmission ΔG_{tr} between the donor and receiver surface (Reproduced with permission from Elsevier Inc.). Data pertain to transmission of *Pseudomonas*, *Staphylococci* and *Serratia* strains from contact lens cases (LC) to soft and hard contact lenses (CL) and from contact lenses to the cornea [48].

Attractive Lifshitz-Van der Waals forces are attenuated in water and higher bacterial transmission is obtained between moist or wetted surfaces in a humid environment than between dry surfaces, such as from dried or moist hands [77] or wetted or dried, bacterially contaminated gloves [17] to test surfaces. This can be fully explained by the Weibull analysis of adhesion forces schematically outlined in Fig. 4, showing that a higher prevalence of weaker donor adhesion forces as under moist or wetted conditions, will yield a higher transmission probability under the influence of a higher, median adhesion force arising from a receiver surface.

4. Structural changes in biofilms during bacterial transmission

Apart from the impact of bacterial cell surface free energy, there is not enough literature available to conclude that specific bacterial strains and species are transmitted more or less than others. In fact, the multitude of different adhesion mechanisms bacteria have at their disposal [64], enables them to transmit themselves to almost any surface, though mostly in small numbers [77], and due to their rapid growth become causative to large problems.

Major difference between effects of transmission on biofilm structure have been described however, between EPS producing and non-EPS producing bacteria especially for biofilms left-behind on donor surfaces after transmission, that are best illustrated in a three-point transmission model [50], outlined in Fig. 6.

The undisturbed biofilm on a donor surface usually has a low volumetric bacterial density. Distances between biofilm inhabitants have been reported to range between 1 and 3 μm [79], while bacterial volume densities have been estimated to be between 0.2 and 0.4 μm^{-3} [50,80,81]. For comparison, the closest hexagonal packing of a 1 μm diameter sphere yields a density of 1.5 μm^{-3}. The low bacterial density in undisturbed biofilms leaves ample voids for compression of biofilm between a donor and receiver surface by an external contact pressure. Water, along with dissolved EPS components will flow out first, as it has the lowest viscosity, followed by more viscous EPS and finally bacteria will redistribute themselves slowly to new, energetically favorable positions. As a net result, bacteria will come closer together and the biofilm will become more compact [50]. There are no experimental methods available to directly measure bacterial densities in a compacted biofilm between a donor and receiver plate [50], but stress-strain diagrams for oral streptococci showed a limited linear elastic trajectory up to a strain of around 0.3, after which the stress required to further compact the biofilm increased exponentially [82].

Separation and detachment occur relatively fast. Biofilms left-behind of non EPS-producing strains on donor surfaces have been found [50] to possess almost two-fold higher volumetric bacterial densities, while biofilm with a viscoelastic EPS matrix restored their density during relaxation to their pre-transmission density due to back-flow of water and EPS (see also Fig. 6). Restoration may however not solely be due to back-flow of water and EPS, but also by a phenomenon called "pressure-induced" EPS production. EPS-producing bacteria transmitted from (sub)monolayers on nanostructured donor surfaces have been found surrounded in EPS patches [4]. Since EPS-production is regulated in part by external forces operating on bacteria [83,84], it was suggested that high local pressures on the bacterial cell membrane triggers opening of efflux pumps resulting in increased EPS production [85,86] during the compression phase of transmission. More extremely, it has been suggested that high local pressures on bacterial cell membranes may compromise the membrane barrier function to cause cell death [87], and this too has been observed during adhesion [88] and transmission [4], especially when involving nanostructured surfaces. Biofilms grown from drinking water systems only relaxed partly to their original thickness after high strain by stresses up to 100 kPa [89]. However, biofilms of *P. aeruginosa* [90] and *S. aureus* [91], demonstrating visco-elastic behavior in stress-strain diagrams, fully relaxed to their original thickness after stress relieve. This supports that EPS

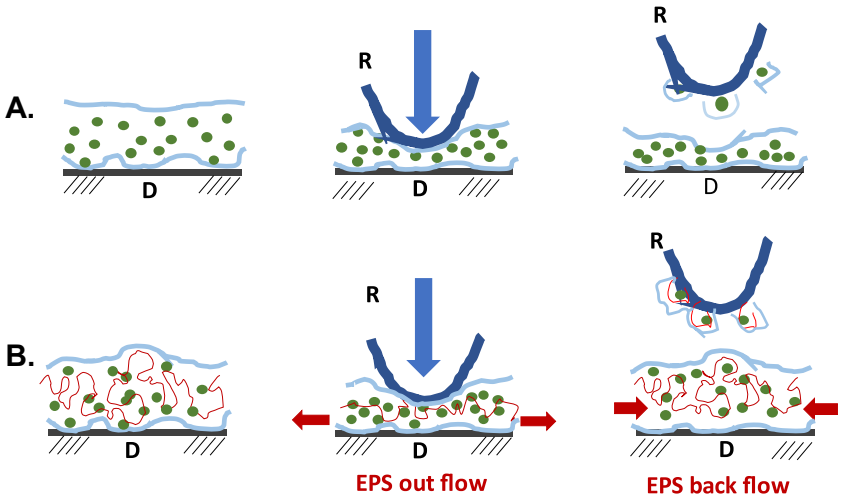

Fig. 6. Three-point transmission model for non-EPS (panel A) and EPS producing bacteria (panel B). Starting with an undisturbed biofilm, the model comprises compaction of the biofilm between the donor (D) and receiver (R) surface, accompanied by EPS outflow when present, followed by and finally relaxation, during which a back-flow of EPS may restore biofilm structure to its pre-transmission state [78].

plays a role in biofilm relaxation after stress application during transmission to its original thickness.

5. The measurement of bacterial transmission

The most distinguishing feature between bacterial adhesion and transmission is the compression of bacteria between two surfaces under an applied contact pressure [3]. Contact pressure applied during experiments has a tremendous influence on the compaction of biofilms left-behind on donor surfaces (see Section 4 of this review). Accordingly during measurement of bacterial transmission, contact pressures should be chosen in accordance with the pressure exerted in the applications aimed for. For reference, holding a coffee cup or using a door handle requires an estimated force of 0.5 kg [92], which roughly corresponds with 5 kPa.

Quantification of bacterial transmission using microscopic means is hampered by the low numbers of bacteria generally transmitted. Culture methods are easier to apply for low bacterial numbers, as particularly occurring during transmission from (sub)monolayers, but culturing only accounts for live bacteria. In addition, if agar culturing is applied, bacteria have to be detached from donor and receiver surfaces, which can be done by scraping or sonication. However, incomplete detachment or bacterial killing during sonication may affect the results and can be avoided by culturing low numbers of bacteria adhering to donor or receiver surfaces in Petrifilm® systems. In a Petrifilm® system, bacteria on a surface are confined between a transparent film containing nutrients and a staining agent and allowed to grow after which colony forming units can be directly counted [93,94]. Transmission of bacteria from multi-layered biofilms can also be studied using culturing methods after detachment and dispersal of biofilms [95,96], but 3D confocal laser scanning microscopy (CLSM) is frequently used as well [97,98]. Different than culturing methods only applicable to live bacteria, 3D-CLSM allows to distinguish between live and dead bacteria after appropriate staining. As a drawback, the relatively small field of view of CLSM makes it difficult to obtain user-independent and statistically significant results. This is particularly troublesome in transmission studies, because the reproducibility of transmission experiments is usually only half of the one that can be achieved in adhesion studies, as transmission involves two processes both possessing large variations, i.e. detachment and adhesion. Optical coherence tomography (OCT) is an emerging method in biofilm analysis and enables reliable measurement of biofilm thickness over a large field of few of several square centimeters [99], but does not allow differentiation of live and dead bacteria [100]. Combination of OCT biofilm thickness measurements with the measurement of bacterial numbers in biofilms (dead and alive) after dispersal, uniquely enables calculation of bacterial volume densities in a biofilm [99,101].

Reproducibility in transmission experiments can be increased by performing a series of consecutive transmissions from the same contaminated donor to different clean receiver surfaces prior to enumeration [92,102]. Since in general low numbers of bacteria are transmitted compared to the total number of bacteria on the donor surface in a single step, the transmission rate *Tr*, defined as the fraction of bacteria that is transmitted from the donor to the receiver in each step, can be assumed to be constant [92]. Accordingly, when constant, the cumulative number of bacteria transferred to the receiver $N_R(t)$ can be calculated as

$$\frac{dN_R(t)}{dt} = Tr * N_D(t) \tag{6}$$

in which $N_D(t)$ is the number of bacteria on the donor left after a total transmission time *t*, i.e. the total time involved in consecutive transmissions. Assuming that transmission is accompanied by a negligible loss in numbers of bacteria

$$N_D(t) = N_{D,0} - N_{Tr}(t) \tag{7}$$

with $N_{D,0}$ the initial number of bacteria on the donor and $N_{Tr}(t)$ is the number of bacteria on the receiver after a transmission time t. Eq. (6) can be solved to yield

$$N_D(t) = N_{D,0}(1 - \exp(-Tr * t) \tag{8}$$

Eq. (8) can be used to calculated transmission rates *Tr* based on the cumulative number of bacteria transmitted over time, with a higher reproducibility than can be obtained in single step transmission experiments.

6. Summary of case studies on bacterial transmission between different materials

In Table 2 an overview is presented of studies carried out on bacterial transmission between surfaces, as confined to studies containing detail

Table 2
Overview of literature studies on bacterial transmission from sub-monolayers (sml) or full grown biofilms from different donor to receiver surfaces, relating to the physico-chemistry summarized in this review.

Donor material	Receiver material	Bacterial strain (sub-)monolayer/biofilm	Key-observations	Reference
Contact lenses	Pig corneas	*P. aeruginosa*/sml *S. aureus*/sml	–Hydrophobic *P. aeruginosa* transmitted in higher numbers than hydrophilic *S. aureus* –Least transmission from hydrophilic and rough lenses	[97]
Contact lenses	Different materials	*P. aeruginosa*/sml *S. aureus*/sml *Serratia marcescens*/sml	–Least transmission to hydrophobic and rough surfaces	[103]
Contact lens cases	–	*P. aeruginosa*/sml *S. aureus*/sml *S. marcescens*/sml	–Weibull analyses of AFM adhesion forces yielded highest transmission probabilities for hydrophilic, polymer-brush coated donor surfaces	[104]
Meat	Stainless steel	*Listeria monocytogenes*/biofilm	–More transmission to stainless steel 304 than to 316	[105]
Smooth and nanostructured silica wavers	Smooth and nanostructured silica wavers	EPS and non-EPS producing staphylococci/sml	–Nanostructured surfaces exert smaller adhesion forces –Less transmission to nanostructured receivers	[4]
Stainless steel	Gloves and theater gowns	*S. aureus*/sml *Propionibacterium acnes*/sml	–Hydrophilic *S. aureus* and *P. acnes* readily transferred to hydrophilic stainless steel –Least transmission from hydrophilic *S. aureus* and *P. acnes* to hydrophobic surfaces	[106]

regarding the hydrophobicity of the donor, receiver and bacterial cell surface, or adhesion forces between them.

Considering that bacterial contamination by bacterial (sub-)monolayers or multi-layered biofilms is inevitable, the overview provided in Table 2 points to two possible pathways for the design of surfaces to which bacteria are less transmitted:

1. polymer-brush coated donor surfaces yield an increased transmission probability, but by the same token can be expected to yield receiver surfaces with less transmission due to their preference to be in a fully hydrated state,
2. nanostructured surfaces present less surface area to bacteria and therewith smaller adhesion forces than corresponding smooth surfaces. Therewith transmission probabilities to nanostructured receiver surfaces become smaller.

Both pathways have not been sufficiently explored however, for practical applications and require further development, e.g. with respect to durability of the surface properties required.

7. Conclusions

Opposing bacterial adhesion and transmission has yielded a better understanding of the physico-chemistry of bacterial transmission. The complexity and experimental problems associated with the study of bacterial transmission between surfaces however, may have discouraged many researchers from doing basic research into transmission phenomena. Yet, in order to develop effective preventive surfaces to prevent bacterial contamination of surfaces through transmission, such studies are direly needed because transmission is fundamentally different from adhesion while yet more occurring in real-life than adhesion. Such a development first of all may require more accurate determination of the adhesion forces and surface energetics that constitute the balance which controls transmission, along with more accurate determination of bacterial transmission itself. Notwithstanding the importance of bacterial transmission in other fields of application, low transmission surfaces, coatings or paints are most direly needed in food industry and in healthcare environments, such as hospitals and nursing homes where transmission of multi-drug resistant pathogens forms a growing problem.

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