Discrimination between random and non-random processes in early bacterial colonization on biomaterial surfaces: application of point pattern analysis

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The dynamics of adhesion and growth of bacterial cells on biomaterial surfaces play an important role in the formation of biofilms. The surface properties of biomaterials have a major impact on cell adhesion processes, eg the random/non-cooperative adhesion of bacteria. In the present study, the spatial arrangement of Escherichia coli on different biomaterials is investigated in a time series during the first hours after exposure. The micrographs are analyzed via an image processing routine and the resulting point patterns are evaluated using second order statistics. Two main adhesion mechanisms can be identified: random adhesion and non-random processes. Comparison with an appropriate null-model quantifies the transition between the two processes with statistical significance. The fastest transition to non-random processes was found to occur after adhesion on PTFE for 2–3 h. Additionally, determination of cell and cluster parameters via image processing gives insight into surface influenced differences in bacterial micro-colony formation.

Keywords: bacterial colonization; point-pattern analysis; bacterial adhesion; biofilm; biomaterials; image processing

Introduction

The formation of biofilms on abiotic surfaces is a persistent and complex phenomenon. In the fields of engineering, natural science and medicine it is very important to understand and control this phenomenon. In particular, implant materials that are intended to substitute human hard and soft tissue are often prone to biofilm formation on their surface (Grinstead 1987; Costerton et al. 1999).

Biofilms are known to initiate an infection at the site of the implant, the so-called ‘biomaterial-associated infection’, frequently necessitating total implant replacement (Gottenbos et al. 2002). Adhesion of bacteria to the biomaterial surface and growth in a monolayer are the first crucial stages in biofilm formation (Katsikogianni & Missirlis 2004). Intervention in the processes at these early stages, eg via the application of suitable surface modifications, has been found to be effective (Renner & Weibel 2011). Extensive studies have been carried out to investigate the influence of surface chemistry (Katsikogianni & Missirlis 2004; Ponche et al. 2010) or surface topography (Medilanski et al. 2002; Giraldez et al. 2010; Ivanova et al. 2010; Singh et al. 2011; Siegismund et al. 2014) on the first stages of biofilm formation, particularly bacterial attachment to the surface. In contrast, relatively few studies focus on the influence of the surface on subsequent bacterial monolayer formation (Barton et al. 1996; Gottenbos et al. 2000; Ploux et al. 2007) or the transition of adhesion mechanisms eg from random to cooperative adhesion (Van der Mei et al. 1993; Beloin et al. 2008). The proliferation of bacterial cells on the surface may be microbiological evidence for a switch from the planktonic to the biofilm lifestyle, and implies a substantial change in the inherent metabolic processes of the cells (Busscher et al. 2012). The initial monolayer growth accompanied by the production of extracellular polymeric substances (EPS) is also a factor in the pathogenesis of a bacterial biofilm. This monolayer is the ‘binding’ layer between the biofilm and the abiotic surface (Gottenbos et al. 2000). Real-time cell tracking studies have been carried out to investigate hypotheses on the attachment of Pseudomonas aeruginosa to surfaces (Gibiansky et al. 2010; Zhao et al. 2013).

In reality, the processes of random adhesion, cooperative adhesion and growth occur concurrently both in vitro and in vivo. Thus, it is difficult to separate these processes quantitatively. Qualitative analysis of bacterial growth kinetics using subjective measures, eg by identifying different slopes of surface coverage evolution curves, could assist in distinguishing different surface induced processes, but this is not a quantitative measure.

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or statistically verified (Ploux et al. 2007). A crucial factor is the time at which the dominant form of bacterial colonization of the surface changes from random attachment to non-random processes. Non-random processes generally lead to an increased local density of bacteria, facilitating local biofilm formation. Quantitative assessment of the time that is required for the transition from random to non-random processes (cooperative adhesion or division) on biomaterials would provide a valuable new measure for determining the performance of a biomaterial. Thus, a suitable biomaterial surface should not only have resistance to bacterial attachment, but also should be assessed for its behavior in monolayer growth and micro-colony formation.

One possible method of achieving such quantification is the use of a second-order statistical method, in particular the pair-correlation function (PCF) or O-ring statistics. This is designed for analyzing the spatial distribution of points within a region of interest and is capable of detecting any significant occurrence of clustered points (Wiegand & Moloney 2004). Originally developed for statistical mechanics (McQuarrie 1975), it is frequently used in ecology to describe spatial patterns (Wiegand & Moloney 2004; Law et al. 2009; Schleicher et al. 2011). In biofilm research, the PCF was introduced in the early 1990s and is occasionally used to study co-adhesion of different bacterial species to biomaterial surfaces (Sjollema & Busscher 1990; Sjollema et al. 1990; Bos et al. 1994; Schillinger et al. 2012).

In the present study the spatial distribution of *Escherichia coli* on four different biomaterial surfaces (titanium dioxide (TiO₂), tissue culture polystyrene (TCPS), polytetrafluoroethylene (PTFE), and glass) was analyzed in a time series during the first few hours after exposing the biomaterial surfaces to the bacterial suspension. Comparison of the evaluated PCF for all acquired points in time with random distributions of the respective point density (null-model) unravels the dependency of the colonization process of *E. coli* on the substratum material. In agreement with a previous work on stainless steel (Hamilton et al. 1995), this study confirms that the initial attachment process of *E. coli* is fully random, even on fundamentally different biomaterials.

The result of the PCF analysis is a quantitative and statistically significant separation of random and non-random surface induced phenomena in the early stages of biofilm formation that has, to the authors’ knowledge, not been achieved in previous work.

**Materials and methods**

**Experimental methods**

Bacterial adhesion kinetics were investigated on TiO₂, TCPS, PTFE and silicate glass surfaces. The points in time chosen for measurements were 1–48 h after exposure of the biomaterial surfaces to the bacterial suspension. *E. coli* EC081 was cultured in a continuous culture (chemostat) that was used for inoculation of a biofilm reactor (non-constant depth film fermenter; nCDFF) to study bacterial adhesion.

Details of the applied experimental protocol can be found in the Supplementary information [Supplementary information is available via a multimedia link on the online article webpage] and in Lüdecke et al. (2013, 2014).

**Microscopy**

A confocal laser scanning microscope (CLSM, Zeiss LSM 510 Meta, Carl Zeiss MicroImaging, Jena, Germany), equipped with an Argon laser (488 nm) and a 63 × NA 1.3 oil immersion lens objective (Zeiss PLANAPOCHROMAT®) was used for imaging the bacterial cells. Applying a 1.5-fold digital zoom resulted in a basic field of view for each image of 71.4 μm × 71.4 μm. CLSM imaging was carried out at five different randomly chosen locations on each sample using three biological replicates, giving a total of 15 different locations of analysis per point in time per material. Since the required quantity for a fully reliable statistical analysis is not obtained directly, for all analytical/mathematical operations that were performed on the measured quantities, a propagation of uncertainty was applied according to Taylor (1997).

**Image processing**

For applying point pattern analysis to the CLSM images, it is necessary to represent the bacterial cells as single points. Thus, specific image pre-processing is necessary, particularly to distinguish between non-spherical bacterial cells as *E. coli* within clusters. All image processing procedures are implemented in the numerical computing environment MATLAB (The MathWorks, Inc., Natick, MA, USA). In the present study, the point approximation is incorporated using the center of mass of the bacterium as a representation, which is a sufficiently accurate method under the present conditions (Wiegand et al. 2006). In Figure 1 an example of the image processing routine is shown.

The first step in the image processing routine is to separate the image into background and bacteria to achieve a binary image. In this study, an automatic thresholding algorithm based on Otsu’s (1979) method was used (see Figure 1b). All bacteria adjoining the image border are not considered in the following processing steps.

It is a complex challenge to specify the centers of mass of single bacterial cells within bacterial clusters.
A topological skeletonizing algorithm for bacterial clusters is incorporated to determine the branching points and subsequently the number of bacteria within the clusters (which is the number of branching points plus one) (Grivet et al. 1999). The centers of mass are then determined by a Gustafson–Kessel clustering algorithm with the determined class size (= number of bacteria) (Gustafson & Kessel 1979).

In order to evaluate the detection accuracy of the number of bacteria within the clusters, three different images at four different points in time per substratum material were compared, with manual counting as the gold standard (see Figure S1).

Laser background scattering occurring during CLSM imaging is different on the investigated materials. Nevertheless the image processing routine is capable of addressing this issue without leading to incorrect calculations (see Figure S2). Furthermore, the performance comparison of the automatic image processing with manual counting shows a mean relative deviation of only 3.8% (see Figure S1).

**Point pattern analysis**

All steps in the point pattern analysis were performed using the programming language R with the Spatstat package (Baddeley & Turner 2005).

PCF characterizes particle density at a particular distance to a reference particle. All identified centers of mass for a single bacterium were passed sequentially, and rings (annuli) with a thickness \( dr \) in an increasing distance \( r \) were generated. A schematic illustration is given in Figure 2.

The PCF is determined mathematically as a ratio of two densities (Sjollema & Busscher 1990):

\[
g(r) = \frac{\rho(r, dr)}{\rho_0} \tag{1}
\]

with:

\[
\rho(r, dr) = \frac{N(r, dr)}{A(r, dr)} \tag{2}
\]

\[
\rho_0 = \frac{N_W}{A_W} \tag{3}
\]

where \( N(r, dr) \) is the number of the bacterial centers (bacterial cells) residing within the respective ring of area \( A(r, dr) \) and \( N_W \) is the number of all bacterial centers within the whole image area \( A_W \).

Generally, \( g(r) \) describes the local bacterial density, in a given radius \( r \) (see Figure 2), relative to the bacterial density within the overall image. Thus, if the density \( \rho(r, dr) \) within one ring is similar to the density in the entire image \( (\rho_0) \), the relation \( g(r) = 1 \) holds and the relative density equals the bacterial density in the whole image.
For $g(r) > 1$, the local bacterial density within the respective ring is higher than that estimated from the overall density. By analogy, for $g(r) < 1$ the distance $r$ is less likely. Edge effects are treated using an isotropic edge correction (Ripley 1977; Wiegand & Moloney 2004).

The PCFs of random and non-random processes are inherently different (Bos et al. 1994). The expected value of the $g(r)$ of a random process is 1, implying that the density of bacteria in the respective ring is similar to that averaged over the whole image (see Equation 1) (Sjöllema & Busscher 1990). For non-random processes, the values of $g(r)$ differ significantly from 1 (Schillinger et al. 2012). The method presented in this study takes advantage of the PCF routine to evaluate bacterial distributions on surfaces over time and to identify the point of transition from random to non-random processes.

**Null-model**

In order to determine statistically significant differences between a completely random distribution and the experimentally observed distributions of bacteria, an adequate null hypothesis has to be established. In the present case, a hard-core effect has been observed due to the reduction of the finite geometries of bacteria to distinct centers. Allowing distances between points in the null-model of less than the least possible distance between bacterial cells (due to their physical appearance) would lead to a falsely decreased point density in the outer rings.

Therefore, a hard-core null-model with an exclusion length of 0.8 μm is used, owing to the minor axis length of the rod-shaped E. coli, which is between 0.6 and 1.2 μm (Mitik-Dineva et al. 2009; Hsu et al. 2013). The exclusion length represents the closest possible distance between two E. coli cells, lying adjacent to each other with their major axes in parallel. The PCFs of the images show no significant difference within the first 0.8 μm, emphasizing the suitability of the selected hard-core distance (see Figure 3). The exclusion length is straightforwardly incorporated to generate the confidence envelopes.

Following Wiegand et al. (2006), 99 point patterns were generated using the null hypothesis and the PCF was calculated. The 95% confidence interval was determined by taking the fifth lowest and fifth highest value for each $g(r)$ (Wiegand & Moloney 2004; Wiegand et al. 2006).

The maximum distance of the PCFs is limited to 10 μm (see Figure 3). Larger bacterial densities (micro-colonies $> 10 \mu m$) are not treatable with the method used due to possible fusion of the micro-colonies. The transition between the prevalence of random and non-random processes occurs at times where the cluster distances were in the range 0.8–10 μm.

The validity and capabilities of the presented point pattern analysis algorithm for the given bacterial densities are discussed on the basis of artificial point patterns that were generated and processed via the analysis pipeline. For details see Supplementary information.

**Summary of the replicates**

The data handling of the analysis from replicated point patterns is often complex (Diggle et al. 2000; Bell & Grunwald 2004). For the pooling of the data from different replicates, a histogram summary has been applied (see Figure 3). The $g(r)$ values for every point pattern (image) that are significantly different from that of the null-model are counted and summarized as fractions (of the pool of replicates) within this histogram. A value of 0.5 corresponds to 50% of point patterns (images) that are significantly different from the null hypotheses in the respective ring. Therefore, the bins of the histogram represent the rings for which the PCF is calculated (see Equation 1 and Figure 2). The width of the rings equals $dr$ (0.35 μm) and the maximum distance $r_{max}$ is 10 μm. An example of a sequence of the point pattern analysis is shown in Figure 3.

**Results**

For early stages of surface colonization, the allocation of bacterial cells on TiO$_2$ cannot be distinguished from an entirely random distribution (Figure 3). After ~9 h, the PCF method detects more neighboring bacterial cells within short distances (1–4 μm) from bacterial cells than predicted by an entirely random distribution (Figure 3, gray envelopes). This indicates a shift to a non-random pattern, where cells begin to arrange themselves in clusters. Evaluation of CLSM images derived from later colonization reveal that also at medium and relatively long distances (1–10 μm) the number of bacterial cells is higher than expected in a random distribution. The fraction of annuli was calculated deviating from the random distribution to illustrate the progressive growth of bacterial clusters (Figure 3, Summary). This procedure was done for all four materials and each time point, and was summarized as an increasing ‘degree of non-randomness’ during the progress of surface colonization (Figure 4, right panels).

Comparison of bacterial colonization kinetics (derived from the CLSM images) and the PCF summary reveals two prevailing processes (observable by different slopes): a non-dominant random adhesion behavior and a dominant non-random behavior. The dominant non-random behavior may result from bacterial growth on the surface, cooperative adhesion or a combination of both (Figure 4).
Furthermore, the inherent differences in the respective adhesion processes on the four substrata are obvious. Random adhesion behavior of *E. coli* cells is observable up to different time points on the tested surfaces ranging from 2 h (PTFE) to 30 h (glass). The change to the predominance of non-random processes (e.g., cooperative adhesion or growth) within the observation period exhibits approximately the same value for TCPS and Ti (∼6–9 h), whereas the transition is faster for PTFE (2–3 h) and much slower for the glass surface (30–38 h) (see Figure 4).

A positive correlation was observed between cluster size (derived from image processing) and the degree of non-randomness of bacterial distribution for all four materials (see Figure 5). The cluster sizes for all points in time range from ∼2–6 bacteria per cluster for glass, TCPS and TiO$_2$ to ∼2–17 bacteria per cluster for PTFE. Accordingly, PTFE exhibits the highest density of bacterial clusters (2.09 bacteria $\mu$m$^{-2}$) compared to glass, TCPS of TiO$_2$ with bacterial densities ranging from 0.74 to 1.07 bacteria $\mu$m$^{-2}$ (see Table 1).

In order to analyze the effect of surface properties on the bacterial size (see Figure 6), bacterial cell sizes were derived from the respective covered surface area which is accessible via image processing (details of the exact image processing method for the determination of the additional parameters shown in Figures 5, 6 and Table 1).
Figure 4. Adhesion kinetics derived from image analysis (left panel) and corresponding summary of PCF statistics (right panel) for four different materials. (a) TiO$_2$; (b) PTFE; (c) TCPS; (d) glass. Boxplots show the degree of non-random distribution of bacterial cells derived from the summary histogram (an example is shown for TiO$_2$ in Figure 3). Note the similar trends in the kinetics and statistical analysis (see text for further details).
can be found in the Supplementary information). Cell sizes are displayed for two different points in time: $t_{\text{initial}}$ represents the bacterial sizes of the two first points in time per material (TiO$_2$, PTFE, TCPS: 1 and 2 h; glass: 2 and 6 h) and $t_{\text{final}}$ represents the bacterial sizes of the single cells for the last point in time for the respective material (TiO$_2$: 18 h; PTFE, TCPS: 13 h; glass: 38 h). Cell sizes for $t_{\text{initial}}$ are not significantly different (Kruskal–Wallis test, $p = 0.41$), whereas significant deviations for $t_{\text{final}}$ are detected (Kruskal–Wallis test, $p < 2.2 \times 10^{-16}$). Finally, a significant decrease was detected in cell size during colonization for all investigated materials, except for glass (Mann–Whitney U test, TiO$_2$: $p < 2.0 \times 10^{-6}$, PTFE: $p < 6.4 \times 10^{-16}$, TCPS: $p < 2.0 \times 10^{-6}$, glass: $p = 0.9$).

The application of CLSM is susceptible to measurement errors in the case of large micro-colonies that exhibit three-dimensional expansion. This is due to the fact that CLSM images represent a near-surface slice of the bacterial micro-colony and thus potentially underestimate the total bacterial colonization. This leads to a measured constant number of attached bacteria in the last points in time (24 and 13 h, respectively; see Figure 4) for TiO$_2$ and PTFE. The method presented in this study is in general tailored for the early stages of biofilm development, i.e., from bacterial adhesion to the formation of micro-colonies, where the transition between prevailing random and non-random processes occurs.

Figure 5. Correlation between the mean size of bacterial clusters and the degree of non-randomness determined via PCF statistics for four different surfaces. (a) TiO$_2$; (b) PTFE; (c) TCPS; (d) glass. Error bars correspond to SD. For details of the image processing to determine the cluster size see Supplementary information.

Table 1. Mean cluster density for the bacteria adhered to the four materials determined via image processing.*

| Material | Mean cluster density (bacteria $\mu$m$^{-2}$) |
|----------|---------------------------------------------|
| TiO$_2$  | 1.07                                        |
| TCPS     | 0.95                                        |
| PTFE     | 2.09                                        |
| Glass    | 0.74                                        |

*For details see Supplementary information: mean values of all points in time and all images.
material (TiO₂, PTFE, TCPS: 1 and 2 h; glass: 2 and 6 h) are shown to have a significant decrease in the bacterial size (see text for further details). The cell sizes shown here correspond to the respective projected cell areas accessible via image processing. The mean bacterial size differs significantly between the observed materials (Kruskal–Wallis, p < 2.2 × 10⁻¹⁶) but not at the initial (Kruskal–Wallis, p = 0.41). All materials, except glass, show a significant decrease in the bacterial size (see text for further details). The cell sizes shown here correspond to the respective projected cell areas accessible via image processing.

**Discussion**

The present study aims at discriminating between prevalently random and non-random processes (e.g., cooperative adhesion and growth) at early stages of surface colonization by applying a novel point pattern analysis algorithm to rod-shaped bacteria adhered to the biomaterial surfaces. Thus, an objective criterion was provided to detect cluster formation. A model system of *E. coli* and four different biomaterials was used. The novelty is the image processing routine with subsequent statistical analysis of spatial point patterns. Distinguishing possible reasons for random or non-random behavior during early bacterial colonization was not the focus of the study, and detailed conclusions about this cannot yet be drawn. In general, two processes are most likely to cause non-random behavior: growth of bacteria at the material surface, and preferential adhesion of planktonic bacteria to already attached bacteria. The latter may be induced by quorum sensing, for example.

**Significance of the point pattern analysis for application to early bacterial colonization**

At the early stages of colonization, the calculations for the TiO₂ surface (see Figure 3) show no or only a few rings with small *r* values that do not fall into the range of variations of the random null-model. This is in agreement with results in the literature that identified initial adhesion processes of bacteria to solid surfaces to be random in colloid systems (Beloin et al. 2008). Later stages of colonization show an increasing fraction of rings that cannot be described by the random null-model, indicating non-random processes.

**Dependency of the transition behavior with respect to material properties**

With the method presented here it is possible to access quantitatively the transition from random to non-random processes on different classes of biomaterials. Gottenbos et al. (2000) described the dependency of the transition for one class of biomaterials (polymeric), considering the change between bacterial adhesion (random) and growth (non-random) by applying real-time image analysis to parallel plate flow chamber experiments. Gottenbos et al. (2000) showed that the growth parameters for *Staphylococcus epidermidis*, eg, generation time, depend on the chemistry of the polymeric surface. Thus, the hydrophobicity of the surface influences the growth of micro-colonies: increasing the hydrophobicity of a surface accelerates bacterial growth (Gottenbos et al. 2000). In the present study it was also found that hydrophobic surfaces accelerated the transition from random to non-random behavior. The water contact angles of the material surfaces, which are a measure of hydrophobicity, were: 113.2° ± 0.8° (PTFE); 88.0° ± 1.8° (TCPS); 74.3° ± 4.9° (TiO₂); 32.7° ± 2° (glass) [for further details of the surface characterization see Lüdecke et al. (2014)]. As mentioned above, the transition between random and non-random processes occurs earlier for more hydrophobic surfaces: after 2–3 h for PTFE; 5–7 h for TCPS; 6–9 h for TiO₂ and 30–38 h for glass (see Figure 4). This is in good agreement with other experimental studies showing a similar qualitative trend (Tegoulia & Cooper 2002; Parreira et al. 2011).

As mentioned earlier, a detailed analysis of the underlying mechanisms responsible for the shift from random to non-random behavior is not the aim of the study, but possible general aspects are discussed briefly, as follows. Differences in the hydrophobicity of the surfaces are an explanation for the different transition point in time between random and non-random behavior. As shown by different studies, the binding forces of bacteria to hydrophobic surfaces are usually stronger than to hydrophilic surfaces (Boks, Busscher et al. 2008, 2009). Consequently, the attachment of bacteria to hydrophobic surfaces tends to be irreversible and the bacteria are immobile, showing less bond-maturation (Boks, Norde et al. 2008, 2009). Following Busscher and Van der Mei (2012), hydrophobic surfaces can provide a better ‘force
Biological implications of parameters derived from point pattern analysis and image processing

Recent studies of biofilm formation on engineered surfaces suggest that the spatial distribution and the size of micro-colonies are crucial for the development of mature biofilms (Gu et al. 2013; Permi & Prokopovich 2013). Shorter distances between the micro-colonies are expected to accelerate biofilm formation (Gu et al. 2013). The present results support these findings: surfaces with a high number of attached bacteria and a high number of clusters (eg PTFE) show a faster transition from random to non-random processes, which would also result in accelerated biofilm formation (see Figures 4 and 5). Additionally, the dynamics of the increase in the cluster size is in good agreement with data derived from the PCF. In addition to second order statistics, image processing was applied to obtain information regarding the morphology of the bacterial cells and clusters (see Table 1 and Figure 6). Interestingly, the mean cell size of bacteria differs on the investigated surfaces, especially for the late time points (see Figure 6).

The design of the present experiment (continuous bacterial culture) allows the generation of planktonic cells in a similar physiological state, ie with similar morphology, as a starting point for the adhesion experiments (Lüdecke et al. 2014). This is consistent with the image analysis showing that the size of attached single cells is not statistically different at early points in time (see Figure 6). Accordingly, the substratum chemistry and topography are the driving forces for the change in cell morphology. In the present study, the cell size at the latest point in time on the polymeric substrate (TCPS: 0.86 μm²; PTFE: 0.64 μm²) is smaller than on all other substrata (TiO₂: 0.93 μm²; glass: 1.21 μm²). Differences in cell size have been observed for different substrata (Mitik-Dineva et al. 2009; Hsu et al. 2013). The distinct differences in cell morphology may arise as a response to different stress states on the different substrate materials, triggered eg by differences in nutrient availability at the surface, or by differences in the forces acting on the bacterial cells (see section above) (Young 2006). For Helicobacter pylori, the cell shape is significantly different when cells adhered to metallic or polymeric surfaces, and also differs between a spiral shape on hydrophobic self-assembled monolayers (SAMs) and a coccoid shape on hydrophilic SAMs (Azavedo et al. 2007; Parreira et al. 2011). In addition, image analysis of attached Acinetobacter sp. on glass surfaces shows a dependency of cell size on nutrient availability, with smaller cell sizes during starvation (James et al. 1995). A general correlation regarding the type of surfaces on which the respective cells are more viable has to be the topic of further studies. The correlation between bacterial cell sizes at t_final and the hydrophobicity of the material is obvious: the more hydrophilic the material the larger the cell size of single bacterial cells. This emphasizes the effect of the different forces (which are higher on hydrophobic surfaces) on the cell response and cluster density (see Table 1 and Figure 6). This bacterial density is also distinctly correlated with resistance to antibiotic treatment and immune defense (Gristina 1987; Stewart & Costerton 2001; Davies 2003). Furthermore, the difference in morphology of bacterial cells and micro-colonies (size, density) indicates that the success of antibiotic treatment is likely to be dependent on the material where the biofilm is established.

As discussed by Van der Mei and Busscher (2012), bacterial cell heterogeneity is a possible cause for different cell morphologies being present on different biomaterials. Especially on PTFE, low cell size was found to be accompanied by a large number of bacteria per cluster, leading to high cluster densities (see Figure 5 and Table 1).

The results clearly show that not only are bacterial adhesion and the subsequent build-up of micro-colonies influenced by the respective substratum, but also the transition points in time between both these processes. Accordingly, the novel point pattern analysis method introduced in this study indicates a suitable time frame for the application of antibiotic agents for the treatment of biofilms.

Summary

With the application of point pattern analysis and PCFs, random adhesion and non-random processes (cooperative adhesion or growth) during the early stages of bacterial biofilm formation are separated. Random adhesion is followed by non-random processes for all investigated materials. The authors were able to characterize the transition between both processes quantitatively which had, to their best knowledge, not been accomplished in
previous studies. Thus, an objective criterion for distinguishing random from non-random processes is provided. Furthermore, image processing revealed differences in cell and cluster morphologies on the different biomaterials. Together with the differences in the change of the surface colonization mechanism, this has important consequences for successful treatment of biomaterial-associated infections of implants by antibiotics.

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