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Quantification of the interaction between biomaterial surfaces and bacteria by 3-D modeling

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ABSTRACT

It is general knowledge that bacteria/surface interactions depend on the surface topography. However, this well-known dependence has so far not been included in the modeling efforts. We propose a model for calculating interaction energies between spherical bacteria and arbitrarily structured 3-D surfaces, combining the Derjaguin, Landau, Verwey, Overbeek theory and an extended surface element integration method. The influence of roughness on the interaction (for otherwise constant parameters, e.g. surface chemistry, bacterial hydrophobicity) is quantified, demonstrating that common experimental approaches which consider amplitude parameters of the surface topography but which ignore spacing parameters fail to adequately describe the influence of surface roughness on bacterial adhesion. The statistical roughness profile parameters arithmetic average height (representing an amplitude parameter) and peak density (representing a spacing parameter) both exert a distinct influence on the interaction energy. The influence of peak density on the interaction energy increases with decreasing arithmetic average height and contributes significantly to the total interaction energy with an arithmetic average height below 70 nm. With the aid of the proposed model, different sensitivity ranges of the interaction between rough surfaces and bacteria are identified. On the nanoscale, the spacing parameter of the surface dominates the interaction, whereas on the microscale the amplitude parameter adopts the governing role.

1. Introduction

Today's advanced medicine relies increasingly on implants and the according functional biomaterials for restoring body functions or replacing soft and hard tissues. Despite all research efforts on the material's side, a relatively high number of so-called "biomaterial-centered infections" occur post-surgically and represent a fundamental challenge in biomaterials science [1,2]. The infections are primarily initiated by the adhesion of bacteria onto a biomaterial's surface, eventually leading to the formation of a bacterial biofilm [3,4]. In the majority of cases, the infection ultimately requires surgical revision and removal of the implant in order to cure the infection. Additionally, the probability for reinfection is significantly increased after a previous infection [5]. The adhesion of bacteria onto a biomaterial surface is the first crucial step in the process of biofilm formation but the details are still not fully understood [6].

The interaction of bacteria with the surface of a biomaterial depends on various properties, such as the surface energy [7], the surface chemistry [8,9] and the surface topography [10]. An elegant method for controlling the bacteria/surface interaction is to adjust the surface topography. This can be used to optimize the biomaterial's properties without the need to alter the chemistry by additional processing steps, such as ion implantation or coating. Chemical surface treatments bear the risk of a shortened application cycle due to mechanical instability. It is expected that topographical surface features with a length scale comparable to that of the bacteria (mostly between 0.5 and 5 μm) have a greater effect on the adhesion process than topographical features in the nanometer range, owing to the limited sensing capability of a single bacterial cell [11]. This is confirmed by available experimental results on the influence of surface roughness and surface morphology on bacterial adhesion to biomaterials [12–20]. The studies mentioned document an increasing total number of bacterial cells adhering to the surface with increasing surface roughness on the micrometer scale [12,15,17]. In contrast, on the nanometer scale the opposite effect, i.e. a decreasing total number of adherent bacterial cells with increasing surface roughness, has been found [16,19]. Current models do not reproduce this ambivalent, length-scale-dependent effect of surface topography.

Surfaces with local depressions (dimples, cavities) of dimensions similar to those of the bacteria exhibit enhanced and preferential adhesion of bacterial cells in these depressions [21,22], while 1-D structures, e.g. periodic scratches on the surface, result in the alignment of both spherical and non-spherical bacteria [23].

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Especially for spherical bacteria, e.g. *Staphylococcus* spp., the retention is attributed to the increase in contact area between the bacterium and the surface [23].

In recent years, several experimental studies have been carried out to precisely link topographic and roughness parameters to the cell/surface interaction [8,24–27]. The authors of these studies conclude that accurate analysis of the influence of surface roughness on the adhesion of bacterial and mammalian cells requires more than a single statistical parameter, e.g. average random arithmetic height or root-mean-square roughness. This is further substantiated by results in different research fields, e.g. optics, where surfaces are routinely characterized using several parameters that contain both spacing and amplitude profile information in order to correctly describe the overall surface topography [28–30].

Quantitative experimental assessment of the effects of varying the spacing and amplitude parameters on bacteria/surface interaction is tedious, and determining all of the relevant parameters comprehensively will rarely be undertaken. The goal of the present work is to assess the influence of surface topography on the adhesion of bacteria to biomaterial surfaces by theoretical modeling to gain insight into the general relations that are at present beyond the reach of purely experimental approaches due to methodical and time restrictions. The following fundamental questions are addressed by the model introduced here:

(1) Which parameters of the surface features influence the interaction and the attachment process of bacteria with biomaterial surfaces most strongly?
(2) What are the differences of the influence of structured surfaces on the nanometer and micrometer scale?

2. Model description

2.1. Extended Derjaguin, Landau, Verwey, Overbeek (XDLVO) interaction energy

A suitable framework for quantifying the adhesion process is the XDLVO theory [31,32]. Initially designed for calculating the interaction of particles with abiotic surfaces in an aqueous solution, it has also been successfully applied to describe the interaction of bacterial cells with abiotic surfaces [33–35]. According to the XDLVO theory, the total interaction energy between two flat parallel plates consists of three components: the non-retarded Lifshitz van der Waals (LW) energy, the constant surface potential electrostatic double layer (EL) and the Lewis acid–base (AB) interaction energy [32,36]:

$$E_{TOT}^{XDLVO}(h) = E_{FPP}^{LW}(h) + E_{FPP}^{EL}(h) + E_{FPP}^{AB}(h) \quad (1)$$

where h is the distance between the two flat parallel plates.

Further details and parameters contained in these energies can be found in Supplement A.

2.2. Surface element integration (SEI) method

Most calculation methods documented in the literature use the Derjaguin approximation (DA) method to calculate the particular DLVO interaction. The DA method does not address topography at all. There is thus no information to be derived from DLVO combined with the DA on the influence of surface roughness on the bacteria/surface interaction.

To determine the interaction energy between a rough surface and bacteria, the curvature of the surface asperities needs to be considered, especially if the roughness is on the nanometer scale [37]. The SEI method was introduced in the late 1990s to incorporate the effect of curvature into the DLVO interaction energies [36]. By applying the SEI method, the DLVO approach captures interactions between two particles [38], between spherical particles and chemically heterogeneous surfaces [39], between spherical particles and artificially structured surfaces [40,41], and between spherical particles and rough surfaces [37,42,43]. All of the mentioned studies treating the interaction between particles and rough surfaces generate artificial three-dimensional (3-D) surface roughness profiles by assuming protruding and depressing hemispheres with various diameters on flat surfaces. By using cylindrical coordinates for a spherical bacterium, the interaction is described as that of a sequence of differential circular rings with the biomaterial surface (see Fig. 1) [36].

The distance of a circular ring to the surface of the bacterium is defined by:

$$h(r,\theta) = D + a \pm a\sqrt{1 - \frac{r^2}{a^2}} - f(r,\theta) \quad (2)$$

where r is the radius of the circular ring (see Fig. 1), θ is the angle defining the actual position on the circular ring on the bacterium (see Fig. 1), $f(r,\theta)$ is the value of the function from the flat base describing the surface at a position defined by θ and r, a is the bacterial radius and D is the closest distance of the bacterium to the surface.

Extending previous work towards more realistic surface profiles, the surface topography and roughness are not considered here by introducing hemispherical asperities on the surface, but by calculating the interaction with surface morphologies defined by any given function $f(r,\theta)$.

The integral over all circular ring segments $rdrd\Theta$ follows as:

$$U_{SEI}^{3D}(D) = \int_0^{2\pi}\int_0^{a}\left[E_{XDLVO}^{TOT}\left(D + a \pm a\sqrt{1 - \frac{r^2}{a^2}} - f(r,\theta)\right)\right] r dr d\theta \quad (3)$$

The double integral is solved numerically using a Newton–Cotes method, applying Simpson's rule for every component of the interaction energy.

Two types of surfaces are considered in the present work. First, a sine function is implemented as a surface defining function for reproducing 1-D structures (e.g. regular scratches), as described in different previous studies in the literature [21,23]:

$$f(r,\theta) = c \cdot \sin(dr\cos(\theta) + e) \quad (4)$$

where c is the amplitude, d is the frequency and e is the phase shift of the sine function.

In this study we assume bacteria to be rigid spheres: the limited deformability of the bacterial cell wall, especially of staphylococci, is documented in the literature [11]. On the cell wall surface occasionally additional features such as fimbriae or curli are observed [44]. Such extracellular features are not included in the present model; they are, however, expected to have an influence on the interaction between the bacterium and details of the surface topography on the nanometer scale. However, the physico-chemical contributions of the topography are still captured based on averages by the extended DLVO theory, e.g. by the surface charge or hydrophobicity. The interaction energies calculated by this method should correlate strongly with the number of attached bacteria as determined in experimental studies.

The second type of surface considered here is the three dimensional (3-D) random surfaces that are generated using a fast Fourier transform method [45]. The incorporation of this statistical approach using a Gaussian height distribution and specific autocorrelation functions generates a realistic roughness profile [46]. The basic parameters for generating random surfaces are the arithmetic average height R_A and the correlation length c_l of the autocorrelation function. In the present study, without reducing the