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Review article

Bactericidal effects of nanopatterns: A systematic review

Khashayar Modaresifar *, Sara Azizian, Mahya Ganjian, Lidy E. Fratila-Apachitei, Amir A. Zadpoor

Department of Biomechanical Engineering, Faculty of Mechanical, Maritime, and Materials Engineering, Delft University of Technology, Mekelweg 2, 2628CD Delft, The Netherlands

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ABSTRACT

We systematically reviewed the currently available evidence on how the design parameters of surface nanopatterns (e.g. height, diameter, and interspacing) relate to their bactericidal behavior. The systematic search of the literature resulted in 46 studies that satisfied the inclusion criteria of examining the bactericidal behavior of nanopatterns with known design parameters in absence of antibacterial agents. Twelve of the included studies also assessed the cytocompatibility of the nanopatterns. Natural and synthetic nanopatterns with a wide range of design parameters were reported in the included studies to exhibit bactericidal behavior. However, most design parameters were in the following ranges: heights of 100–1000 nm, diameters of 10–300 nm, and interspacings of <500 nm. The most commonly used type of nanopatterns were nanopillars, which could kill bacteria in the following range of design parameters: heights of 100–900 nm, diameters of 20–207 nm, and interspacings of 9–380 nm. The vast majority of the cytocompatibility studies (11 out of 12) showed no adverse effects of bactericidal nanopatterns with the only exception being nanopatterns with extremely high aspect ratios. The paper concludes with a discussion on the evidence available in the literature regarding the killing mechanisms of nanopatterns and the effects of other parameters including surface affinity of bacteria, cell size, and extracellular polymeric substance (EPS) on the killing efficiency.

Statement of significance

The use of nanopatterns to kill bacteria without the need for antibiotics represents a rapidly growing area of research. However, the optimum design parameters to maximize the bactericidal behavior of such physical features need to be fully identified. The present manuscript provides a systematic review of the bactericidal nanopatterned surfaces. Identifying the effective range of dimensions in terms of height, diameter, and interspacings, as well as covering their impact on mammalian cells, has enabled a comprehensive discussion including the bactericidal mechanisms and the factors controlling the bactericidal efficiency. Overall, this review helps the readers have a better understanding of the state-of-the-art in the design of bactericidal nanopatterns, serving as a design guideline and contributing to the design of future experimental studies.

Contents

* Corresponding author.
E-mail address: k.modaresifar@tudelft.nl (K. Modaresifar).

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1. Introduction

Recurrent bacterial infection is one of the major causes of implant failure [1], hugely impacting the patients' quality of life and ultimately resulting in morbidity and even mortality [2]. This type of infection starts off with the attachment of the bacteria to the implant surface, leading to biofilm formation and, thus, high resistance against antibacterial agents [3]. To date, numerous strategies have been proposed to prevent biofilm formation and implant-associated infections. The main working mechanisms of the proposed strategies are preventing bacteria from adhering to surfaces, killing bacteria that manage to attach to the surface, and a combination of both aforementioned approaches. Examples of the first approach are antibiofouling surfaces that are made by altering the chemical and/or physical properties of the surface, thereby making them highly unfavorable for cell and bacteria attachment. The result is a non-adhesive or cell-repellent surface (Fig. 1) [4]. This strategy may be suitable for implants whose integration in the body is not dependent on tissue regeneration. However, many implantable medical devices, in general, and orthopedic implants, in particular, require a substantial amount of tissue regeneration to support osseointegration and implant fixation. It is therefore important that the implant surface supports cell attachment and proliferation while inhibiting bacterial growth [5].

The second approach is based upon surfaces that allow for cell adhesion but kill the bacteria upon contact. They are sometimes called bactericidal surfaces and are the holy grail of implant surface design. The primary working mechanism of bactericidal surfaces is either chemical or physical. Common chemical methods use surface bio-functionalization or surface coatings to enhance the antibacterial properties of the surface. For instance, coatings releasing antibiotics [6–8] and silver ions [9–13] have been shown to be effective in killing bacteria and preventing biofilm formation. However, antibacterial agents such as antibiotics or silver nanoparticles may cause different types of toxicity including cytotoxicity [9,14], nanotoxicity [15], or nephrotoxicity [16]. In addition, continuous low-dose release of antibacterial agents, which is required for long-term protection against implant-associated infections, may cause the bacterial strains to develop resistance against those agents [17]. Furthermore, it has been recently shown that after repeated exposure to silver nanoparticles, some bacterial strains develop resistance against them even without mutations [18].

The above-mentioned limitations of the chemical approach underscore the importance of physical mechanisms to combat implant-associated infections. The fact that physical cues such as substrate stiffness and roughness, or surface micro/nanotopography influence the behavior of both bacterial [19–22] and mammalian cells [23–27], is a relatively recent discovery. Several studies during the past decades have investigated the effects of surface topography

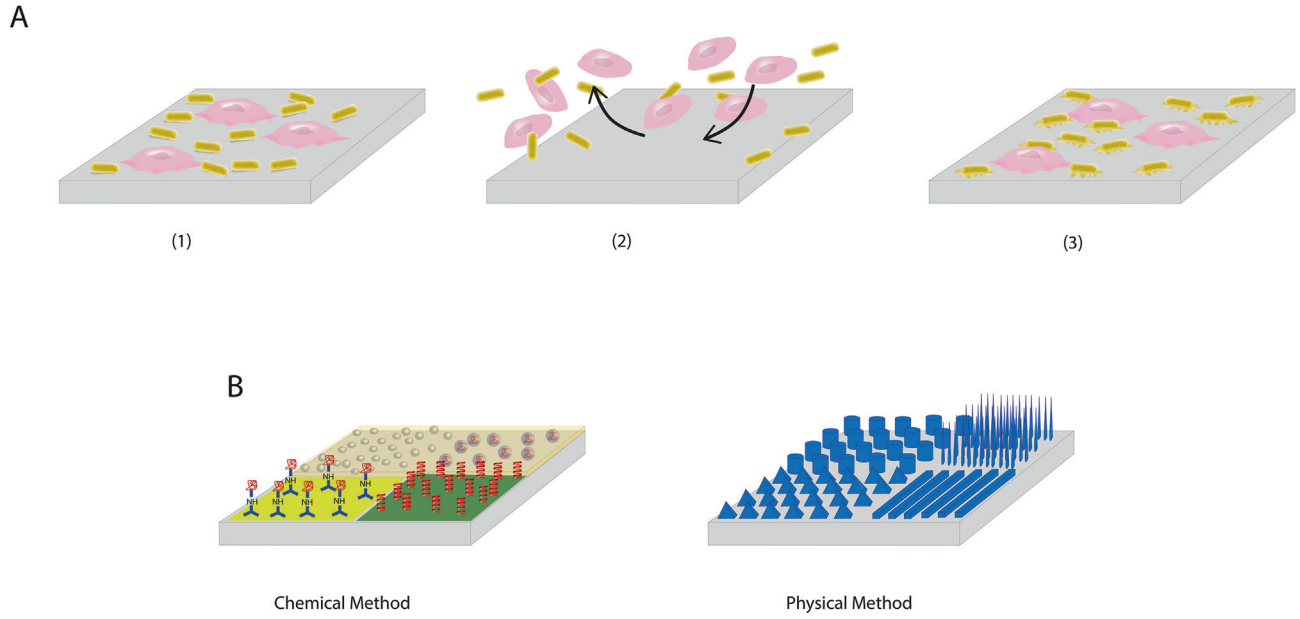

Fig. 1. Different approaches to design antibacterial surfaces. (A) 1. Common surfaces, which were traditionally used in biomaterials engineering, are a favorable place for attachment and growth of both bacteria and human cells; 2. Antibiofouling surfaces have been developed that do not allow microorganisms adhere to the biomaterial surface and prevent possible bacterial infections; 3. Novel desirable advanced surfaces, which are designed and fabricated to kill the bacteria while supporting human cells growth in order to improve the function of implants and tissue engineering scaffolds. (B) Two main approaches in the design of surfaces of advanced biomaterials are chemical and physical modifications. The former is usually associated with coating or chemical grafting of functional groups and/or antibiotics to the surface in order to kill the bacteria. The physical methods, on the other hand, concern with fabricating nanoscale structures with specific dimensions on the surface that could mechanically kill the bacteria through different mechanisms. Recent advances in nanofabrication methods and the problem of antibiotic resistance have made the physical approach more interesting.

on cell attachment, proliferation, and differentiation, as well as bacterial adhesion and motility, revealing the fact that both eukaryote and prokaryote cells could sense the surface topography at both micro- and nano-scales [28–30]. Due to the recent advances in micro- and nano-fabrication techniques, it is now feasible to produce surfaces with arbitrarily complex and precisely controlled surface nanotopography, also known as nanopatterns [29,31–33]. It has been shown that nanopatterns are powerful tools for directing the stem cell fate [34]. Nanopatterns appear in different shapes like nanopillars, nanogrooves, and nanopits with different sizes in height, width, depth, and spacing, which are dependent on the fabrication technique. Subsequently, the feature size modulates the interaction of nanopatterns with cells. Not only could surface nanotopography determine stem cell fate, many studies have shown that high aspect ratio nanopatterns are capable of killing bacteria [35,36] and preventing biofilm formation [37].

An important question regarding the bactericidal behavior of nanopatterns is the optimum design parameters to maximize the bactericidal behavior while minimizing the potential adverse effects such as cytotoxicity. An increasing number of studies have addressed this research questions during the last decade. Given that nature has always been a great source of inspiration for developing advanced materials and systems with a wide range of applications such as self-cleaning surfaces [38], antibiofouling surfaces [39], and reversible adhesive surfaces [40], bio-inspired surfaces have been studied in this context too. For instance, cicada wings are known to be lethal against a wide range of Gram-negative bacteria [41]. Further studies on similar surfaces have led to reproducing nanopatterns of similar size and shape on implantable biomaterials [37,42]. Despite a growing body of knowledge in this area, there is currently no systematic study of the available evidence to understand how the different design parameters of nanopatterns influence their bactericidal behavior. Here, we present a systematic review of the relevant data available in the literature to provide a guideline for designing bactericidal nanopatterns and to contribute towards the development of a quantitative theory of how nanopatterns kill bacteria.

2. Methods

We used Web of Science and Scopus as our primary search databases, while Google Scholar served as a Supplementary Database. Different combinations of the following groups of keywords were searched for: (nanotopography OR nanopattern OR nanotube OR nanopillar OR nanopit OR nanocolumn), (bactericidal OR antibacterial), (bacterial adhesion OR bacterial proliferation OR bacteria), and (mechanotransduction OR mechanosensing). The keyword search resulted in 642 initial hits. Two inclusion criteria were used when screening the abstracts of the articles.

Firstly, the articles should have investigated the antibacterial effects of nanopatterns with controlled or characterized shapes and dimensions. This criterion excluded the studies on surface nano-roughness and the studies in which antibiotic-releasing nanoparticles have been used. Secondly, the studies should have provided evidence of antibacterial activity or at least discuss the possible killing mechanism induced by the nanopatterns. Based on the above-mentioned inclusion criteria, 105 documents were further examined to determine if they meet the following two conditions: 1) the article is an original research paper and not a review paper, book chapter, or a thesis; 2) the effects of nanopatterns are investigated in the absence of antibacterial agents. Moreover, reference tracking was carried out in the full-text of all articles in order to avoid missing any relevant studies. A total of 46 studies satisfied the inclusion criteria and are further discussed in the remainder of this work.

3. Results

The results of the literature search are detailed in Table S1 (see supplementary document) and summarized in this section.

3.1. Literature search output

Only 23 papers provided a comprehensive discussion or clearly presented a hypothesis regarding the possible killing mechanism of the nanopatterns and compared it to other studies. Moreover, Gram-negative and Gram-positive bacteria were respectively used in 34 and 32 studies, and 21 studies used both. Twelve studies also reported the effects of nanopatterns on mammalian cells.

The most common types of nanopatterns were nanopillars (21), nanowires (5), nanocolumns (3), nanopores (3), nanocones (3), spinule-like nanostructures (3), and nanospikes (2). From the 46 studies, 8 investigated nanopatterns found in nature and did not specify any fabrication method. The most common methods for fabrication of the nanopatterns included reactive ion etching (RIE) (9), hydrothermal treatment (5), anodizing (3), chemical etching (2), plasma etching (2), glancing angle sputter deposition (2), electrodeposition (2), and nanoimprint lithography (2). The most common types of materials used for creating the nanopatterns were silicon (13), titanium oxide (TiO_2) (8), titanium (4), poly(methyl methacrylate) (PMMA) (3), zinc oxide (ZnO) (2), and gold (2).

3.2. Effective dimensions of bactericidal nanopatterns

Nanopatterns with a wide range of dimensions were reported in the included studies to be bactericidal (Fig. 2). The dimensions of the nanopatterns were usually presented in terms of height/length (H/L), diameter/width (D/W), and spacing (S).

Nanopillar arrays with $100\ nm < H < 900\ nm$, $20\ nm < D < 207\ nm$, and $9\ nm < S < 380\ nm$ have been found to be effective in killing bacteria. Similar effects were observed for nanocolumns with $250\ nm < H < 478\ nm$, $33\ nm < D < 300\ nm$, and $100\ nm < S < 200\ nm$. Nanocones with the following dimensions were also found to be bactericidal: $100\ nm < H < 350\ nm$, $10\ nm < D < 80\ nm$, and $175\ nm < S < 250\ nm$.

These ranges are somewhat wider when considering nanopatterns in the shape of spikes or spinules. Spikes with $S \approx 220\ nm$, $20\ nm < D < 200\ nm$, and $H > 200\ nm$ (up to $11\ \mu m$) have been shown to display bactericidal characteristics. Natural spinules (mostly found on gecko's skin) and their synthetic replicas have a height in the microscale ($1–4\ \mu m$) range but their diameters and spacing are in the nano and submicron scales (10–85 nm and 500–1000 nm, respectively).

As for nanowires, the reported dimensions are $298\ nm < H < 350\ nm$, $30\ nm < D < 143\ nm$, and $S \approx 77\ nm$. The few studies on nanopores, nanopits, and nanogrooves show that a depth of as small as 2.3 nm may be sufficient for inducing bactericidal behavior provided that it is combined with a diameter of 40–99 nm and a spacing of 70–300 nm.

In summary, most studies report the bactericidal behavior for nanopatterns whose dimensions lie in the following ranges: $100\ nm < H < 1000\ nm$, $10\ nm < D < 300\ nm$, and $S < 500\ nm$.

3.3. Cytocompatibility of nanopatterns

Among the studies (12) that examined the cytocompatibility of surface nanopatterns, the majority (11) found no adverse effects on mammalian cells. For instance, the morphology of human mesenchymal stem cells (hMSCs) remained unchanged and osteoblasts proliferated on Ti nanocolumns ($250\ nm < H < 500\ nm$ and

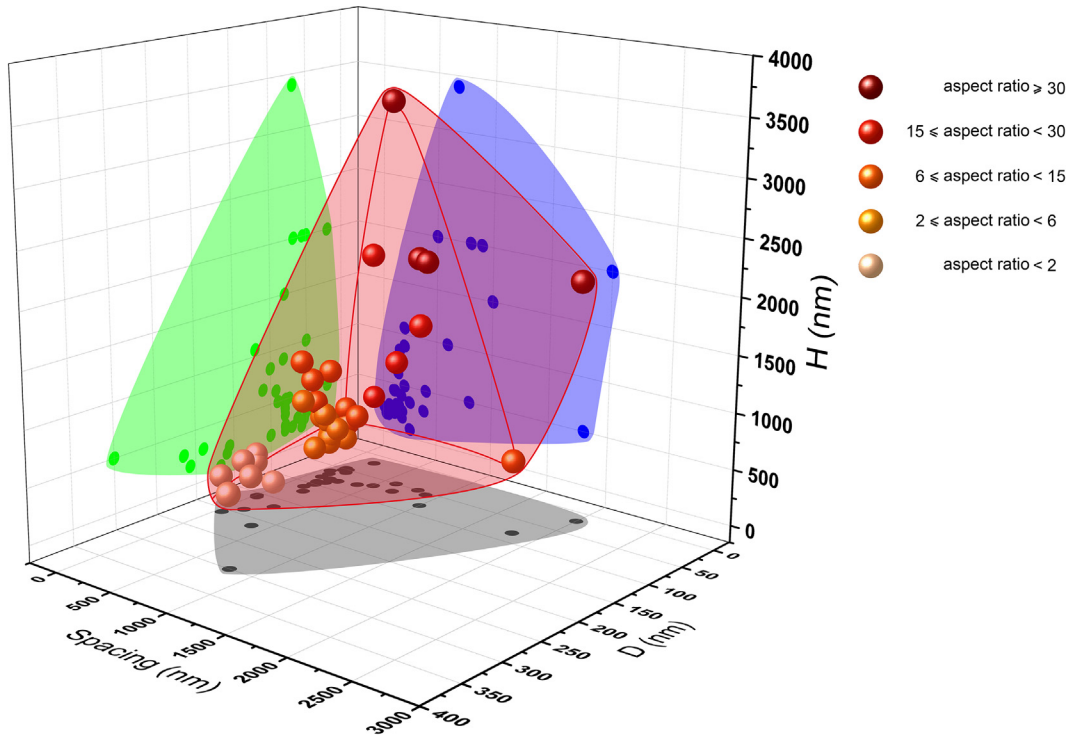

Fig. 2. Effective dimensions for bactericidal nanopatterns. The red area indicates the range of dimensions in which the nanopatterns show bactericidal activity. The majority of bactericidal nanopatterns reported in the literature (colored bullets) have a height of 100–500 nm, a diameter of 10–300 nm, and a spacing of 10–380 nm. Different colors of bullets show the aspect ratio of nanopatterns reported in each study. The green, blue, and grey projections enable comparison between those three parameters. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

30 nm < *W* < 60 nm). Growth and proliferation of other cell types such as keratinocyte, fibroblast, and human dental pulp stem cells on different types of nanopatterns have been also reported in the literature [43–45]. The dimensions of the nanopatterns were in most cases as follows: diameter of 10 to 100 nm and heights of 200 to 650 nm. One study [46] reports that nanopatterns with extremely high aspect-ratios (>200) may kill mammalian cells in addition to bacteria.

4. Discussion

The results of this systematic review show that nanopatterns with a relatively wide range of design parameters could exhibit bactericidal behavior. The included studies have, in many cases, investigated the bactericidal properties of the surfaces using scanning electron microscopy (SEM) to observe any drastic changes or deformations in the morphology of the bacteria, the disruption of the cell, and any other signs of damaged or dead bacteria. To quantify the bactericidal efficiency, most studies count the colony forming units (CFU) and use live/dead staining. The former determines the number of viable bacterial cells able to form a colony after being exposed to the nanopatterns, and the latter distinguishes between the viable and dead bacterial cells based on the integrity of the membrane [47,48]. As most studies found no adverse effects of nanopatterns on mammalian cells, there seems to be a large window within which bactericidal nanopatterns could be designed without negatively influencing the attachment and proliferation of host cells that are required for tissue regeneration and integration of the implant in the human body. Nevertheless, most of the included studies investigate the bactericidal activity within the first 24 h *in vitro*. It is therefore not completely clear what happens to the bacteria after they are killed and what will happen if the surfaces are constantly exposed to subsequent bacteria. Unlike the "kill and release" surfaces [49], the remnant components of the dead bacteria may negate the long-term functionality of the nanopatterns. Further *in vivo* studies are, thus, required to understand if the immune cells can effectively clean up the surface and whether the surface will maintain its bactericidal activity in the long-term [50]. On the other hand, the antibacterial properties of the biomaterials surfaces may be most crucial in the first few hours after implantation where the "race for the surface" [50] is ongoing. If bactericidal surfaces deter the bacteria in those first few hours, the host cells are more likely to win that race after which there will be less need for antibacterial protection. Indeed, it has been shown that mammalian cells can win the race and dominate the surface in long-term. For example, Pham et al. have shown that eukaryotic cells could grow and proliferate on a pre-infected nanopatterned surface right after that surface had inhibited bacterial growth on it [45]. The fact that mechanosensing pathways could be different in eukaryotic and prokaryotic cells [28,51], opens the way to design nanopatterned surfaces which selectively allow eukaryotic cells to survive [45]. Nanopatterns which initially eliminate the bacteria and subsequently promote host cells to attach and proliferate on the surface may therefore be able to deliver long-term benefits.

Despite the advances in micro/nanofabrication techniques, it is very challenging to create nanopatterns on 3D-shaped devices as the current patterning techniques are mostly only applicable to flat surfaces. Novel strategies have been therefore proposed to create 3D structures from flat sheets that are first ornamented with nanopatterns and are then (self-)folded into complex 3D shapes using origami-based approaches [31]. This could be promising for translating bactericidal and osteogenic nanopatterns to clinical use.

4.1. Bactericidal mechanisms of nanopatterns

The interactions between surface nanopatterns and bacteria are multi-faceted, making it difficult to fully delineate the role of various influencing factors. As a consequence, the exact killing mech-

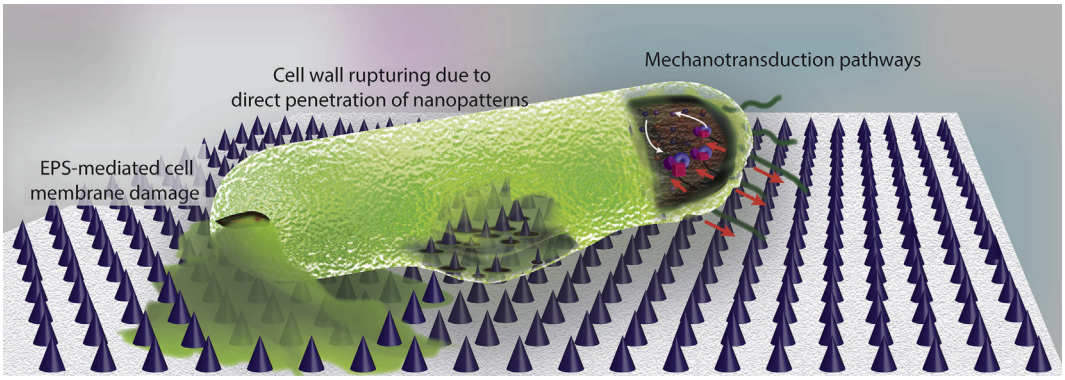

Fig. 3. The main bactericidal mechanisms of nanopatterns. While the commonly believed theory is that bacterial cell wall is ruptured by penetration of high aspect ratio nanopatterns, there are a few studies suggesting that EPS plays a key role in this regard. It has been shown that the strong attachment of EPS to the nanopatterns and the attempts of bacteria to move away from the unfavorable surface leads to cell membrane damage. Moreover, mechanotransduction pathways in which the mechanical forces affect the metabolomics and the genomics of bacteria could be possible mechanisms of bacteria death on the surface.

anism and the role of various factors in regulating the bactericidal behavior of nanopatterns remain controversial [35,52]. Notwithstanding the disagreements, most researchers agree that mechanical deformation in general and the rupture of cellular components such as cell wall due to large deformations and the penetration of high aspect ratio nano-features play a key role in this regard (Fig. 3) [36,43,53–56]. The optimum shape and dimensions of nanopatterns for maximizing the bactericidal behavior are, nevertheless, unknown (Fig. 2).

When trying to understand the antibacterial behavior of nanopatterns, it is important to separate the effects of surface chemistry from those of nanopatterns. For example, naturally occurring bactericidal surfaces are often hydrophobic in nature and exhibit low surface energy, which varies with the dimensions of the nanopatterns [57]. To isolate the effects of nanopatterns and eliminate the effect of surface chemistry, nanopatterned surfaces have been coated with gold [58]. The coated surfaces exhibited similar antibacterial behavior, suggesting that the bactericidal behavior is physical in nature. Moreover, hydrophilic surfaces are shown to exhibit bactericidal properties as well [58]. In the cases where the nanopatterns are made from materials that possess intrinsic antibacterial activity, e.g. TiO_2 or ZnO, it may be challenging to isolate the effects of nanopatterns from those of the material itself. Moreover, the material and nanopatterns may work synergistically to kill bacteria. A number of studies that satisfied the inclusions criteria and are therefore included in this review have used nanopatterns based on TiO_2 or ZnO. Given the fact that many studies that use TiO_2 nanopatterns coat them with gold, the intrinsic antibacterial effects of the material are not expected to have played a major role in those studies. However, further systematic studies are needed to fully understand the isolated and synergistic effects of nanopatterns and TiO_2 on bacteria, as not enough control surfaces have been used in most of the included studies. Concerning ZnO, it has been shown that the ZnO nanopillars produced on different types of substrates kill the adhered bacteria by mechanical rupturing with no significant difference in the killing efficiency [59]. Unlike other substrates, however, the ZnO nanopillars made on a zinc substrate were also capable of killing non-adhered bacteria through release of high concentrations of superoxide radicals, which were generated by electron donation from zinc [59]. Other studies in the literature have also suggested that the antibacterial activity of ZnO nanowires could be partially attributed to the release of zinc ions [60,61]. Taken together, these results suggest that when TiO_2, ZnO, or other types of materials that possess intrinsic antibacterial properties are used for creating nanopatterns, multiple control surfaces should be included in the study to enable separating the effects of nanopatterns from those of the material itself and to quantify any synergistic effects that may be present.

4.2. The role of surface affinity and surface adhesion

The high aspect ratio features found in nanopatterns could also influence the affinity of microorganisms to the surface [62]. Interestingly, the level of bactericidal behavior is found to increase with the level of adherence of the microorganism to the surface [62]. Another study has shown that the susceptibility of different types of bacteria to mechanical rupturing by nanopattern varies with their stage of maturity [55]. For example, the nanopillars found on the wing of *Calopteryx haemorrhoidalis damselfly* were deadliest against young *Staphylococcus aureus* and mature *Pseudomonas aeruginosa* cells [55]. This behavior was explained to be related to the higher affinity of the bacteria to the surface in those specific stages of their life [63]. These studies suggest that a high degree of adherence to the nanopatterned surface is the first necessary step to have the bacteria killed on the nanopatterns. In contrast to these findings, nanocolumns produced on titanium surface were found to decrease the surface covered by bacteria, as well as biofilm formation [37]. Further studies revealed that the dimensions of nanopatterns [64] and the contact time [65] both affect the number of bacteria attached to the surface. More importantly, nanopatterns mainly affect the adhesion forces and not necessarily the number of adhered bacteria [66].

4.3. The effects of other nanopattern design parameters

In addition to the design parameters considered here (i.e. height, diameter, interspacing), there are a number of other parameters that may influence the bactericidal behavior of nanopatterns. Fisher et al. found that a surface patterned non-uniformly (with more varying dimensions) is more lethal to the bacteria [67]. There is, however, a need for more conclusive data with a wider range of studied parameters (shapes, dimensions, etc.), as other studies have shown that disorganized nanopatterns are not as effective as the organized ones [61]. A few studies have also investigated the effects of compaction and density of nanopatterns on the bactericidal activity. Linklater et al. showed that smaller and more compact nanopillars on black silicon surface are more effective in killing both Gram-negative and Gram-positive bacteria as compared to larger and more separated nanopillars on the same surface [68]. Furthermore, it has been recently shown that optimizing the density of nano-features could play a crucial role

in the killing efficiency of nanopatterns against *Staphylococcus aureus* [69].

4.4. The effects of bacterial-dependent characteristics

The intrinsic characteristics of bacteria could also influence the interactions between bacteria and nanopatterns [17,70]. For instance, the bactericidal behavior of nanopatterns has been shown to be dependent on the motility of the bacteria with highly motile bacteria being killed more efficiently [71]. Hasan et al. demonstrated that nanopatterns hold on to the bacterial membrane of *Escherichia coli* and cell wall of *Staphylococcus aureus*. Due to bacterial motility, the membrane/cell wall is being increasingly stretched and eventually permanently deformed, thereby leading to cell death [46]. Moreover, bacteria with different morphologies exhibit different degrees of adhesion to the nanopatterns, as observed for *Staphylococcus aureus* (coccoid-shaped) and *Pseudomonas aeruginosa* (rod-shaped) when cultured on the same nanopatterned surface [72].

There may be additional effects associated with the size of the bacteria [67,73]. According to a theory presented by Li et al. [73], larger bacteria cells (i.e. larger than the spacing between the nanopatterns) may get penetrated and ruptured, whereas those that are smaller than this dimension, interact with the side edges of the nano-features. They may be therefore either stretched due to the gravity and adhesion forces or compressed between the bases of the nanopatterns. In the case of spinule-like structures, there may be different stiffness values along the height of a single nano-feature. Those stiffness variations may further affect the viability of the bacteria [73].

4.5. The role of extracellular polymeric substance (EPS)

The role of EPS in regulating the bactericidal effects of nanopatterns has been highlighted in some recent studies [52]. It has been, for example, shown that at least some bacterial strains secrete strongly adherent EPS when subjected to the nanopillars of the dragonfly wing [52]. Once the bacteria find the surface unfavorable and try to move away, EPS anchorage causes cell wall rupture and cell death [52]. The findings of this study have highlighted the role of EPS in the bactericidal behavior of nanopatterns [52]. Linklater et al. performed a study on the bactericidal mechanism of black silicon, which has been shown to be comparable to the dragonfly wing in its killing efficiency against Gram-negative and Gram-positive bacteria [35]. The results showed that the bacteria are killed within 3–5 min of contact with the nanopatterns, which is not enough for bacteria to produce and secrete EPS [35]. Moreover, neither the affinity of cells with the surface nor the motility of the bacteria influenced the killing efficiency of the nanopatterned surface [35]. The key factor in determining the bactericidal behavior was the height of the nanopatterns [35].

Even though only one study has so far demonstrated the role of EPS in regulating the bactericidal behavior of nanopatterns, it may have more far-reaching effects than currently thought. Previous studies have shown that pressure-induced EPS production leads to higher levels of bacterial death since the membrane efflux pumps open during this process and impair the membrane barrier function [66]. Bacterial EPS mainly consists of proteins, extracellular DNA, and polysaccharides, which all play various crucial roles in the development of the biofilm including forming the 3D architecture of the biofilm, protecting it against environmental factors, and facilitating cell-cell signaling [74,75]. For instance, studies on the EPS composition in *Pseudomonas aeruginosa*, widely studied as the model biofilm-forming organism, have shown that it is mainly composed of two polysaccharides, namely Pel and Psl. Pel is highly involved in the adhesion of bacteria to the surface and maintaining the cell-cell interactions in the bacterial biofilm, as well as providing a level of protection against aminoglycoside antibiotics [74,76,77]. Manipulating the Pel secretion is proposed to be effective in disrupting the biofilm [76]. Moreover, Pel is associated with making the adhesion forces short-ranged and symmetric [78]. Therefore, it is plausible to hypothesize that mechanical forces which disrupt the function of Pel, eventually kill the bacteria through a direct mechanotransductory pathway. The indirect mechanotransduction pathways could also be responsible for the bacterial death, as nanoscale topography may affect the genomics and proteomics of the bacterium [79]. Further studies are therefore required to determine the exact mechanisms through which nanopatterns kill bacteria and the role of EPS.

4.6. Interactions of mammalian cells with the nanopatterns

There are differences in the ways bacteria and mammalian cells attach to surfaces and sense them. While bacteria form a community within the EPS to live on the surface, eukaryotic cells are able to adhere to the surface as single cells [29]. Unlike bacteria, this adhesion is always mediated through the extracellular matrix (ECM) [29]. Cellular features to probe the surface also differ between these two types of cells. The adhesion of mammalian cells to the ECM or other nanotopographical structures is mediated by integrins, which form dynamic adhesion structures known as focal adhesions [34]. It has been shown that nanotopography could alter the expression of integrins and focal adhesions signaling, which finally influences the cytoskeletal structure [80] indicating activation of mechanotransduction pathways. Moreover, the design parameters of nanopatterns (e.g. height) have been shown to affect the size and density of the focal adhesions in MSCs [81,82]. Bacterial cells are much smaller than mammalian cells and due to their more rigid cell wall, they are less deformable. These differences affect the sensing mechanisms and the following mechanotransduction pathways induced by nanotopographical features whose dimensions are comparable with the size of the bacteria [29]. Therefore, those nanopatterns, which are lethal to the bacteria, could possibly trigger direct or indirect mechanotransduction pathways within mammalian cells affecting their function. For instance, it has been shown that the osteogenic differentiation of MSCs is sensitive to a variety of factors including the spatial arrangement of the nanopatterns and their shapes [83,84]. Moreover, an optimum height of nanopillars could be identified yielding the highest osteogenic marker expression in MSCs [28]. Thus, using nanopatterns with specific designs could be a promising tool in directing stem cell fate.

Although more studies on nanotopography-induced cellular responses and mechanotransduction pathways have been carried out on mammalian cells than bacterial cells, the exact molecular mechanisms and outcomes are yet to be discovered, as these pathways are complex and involve a large number of biomolecules and signals [34].

5. Conclusions

We systematically reviewed the studies on the bactericidal effects of nanopatterned surfaces. Different types of nanopatterns with heights of 100 nm to >900 nm, diameters between 10 and 300 nm, and interspacings of <500 nm have been reported in the literature to exhibit bactericidal properties. Most of the studies that also examined the impact of nanopatterns on the mammalian cells found no evidence of adverse effects with the only exception being nanopatterns with extremely high aspect ratios.

Controversy exists over the exact killing mechanism of nanopatterns and the factors controlling the bactericidal efficiency.

While the main mechanism is thought to be mechanical in nature and associated with the rupturing of the bacterial cell wall by high aspect ratio nanopatterns, some studies suggest that the EPS produced by the bacteria and the intrinsic properties of bacteria such as motility and size play a role as well. Overall, high aspect ratio nanopatterns could be considered as an effective tool for killing bacteria, especially because they achieve this goal without any need for chemical agents or antibiotics, thereby offering an alternative route for the design of the next generation of implantable medical devices.

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Conflict of interests

The authors declare no conflict of interests.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.actbio.2018.09.059.

References

[1] W. Zimmerli, A. Trampuz, P.E. Ochsner, Prosthetic-joint infections, N. Engl. J. Med. 351 (16) (2004) 1645–1654.
[2] J.M. Steckelberg, D.R. Osmon, Prosthetic joint infections, Infections Associated with Indwelling Medical Devices, Third Edition., American Society of Microbiology, 2000, pp. 173–209.
[3] T. Peel, Introduction to Prosthetic Joint Infection, Prosthetic Joint Infections, Springer, 2018, pp. 1–4.
[4] J. Hasan, R.J. Crawford, E.P. Ivanova, Antibacterial surfaces: the quest for a new generation of biomaterials, Trends Biotechnol. 31 (5) (2013) 295–304.
[5] Y. Wang, G. Subbiahdoss, J. Swartjes, H.C. van der Mei, H.J. Busscher, M. Libera, Length-Scale Mediated Differential Adhesion of Mammalian Cells and Microbes, Adv. Funct. Mater. 21 (20) (2011) 3916–3923.
[6] S. Bakhshandeh, Z. Gorgin Karaji, K. Lietaert, A.C. Fluit, C.E. Boel, H.C. Vogely, T. Vermonden, W.E. Hennink, H. Weinans, A.A. Zadpoor, Simultaneous Delivery of Multiple Antibacterial Agents from Additively Manufactured Porous Biomaterials to Fully Eradicate Planktonic and Adherent Staphylococcus aureus, ACS Appl. Mater. Interfaces 9 (31) (2017) 25691–25699.
[7] B. Wang, H. Liu, Z. Wang, S. Shi, K. Nan, Q. Xu, Z. Ye, H. Chen, A self-defensive antibacterial coating acting through the bacteria-triggered release of a hydrophobic antibiotic from layer-by-layer films, J. Mater. Chem. B 5 (7) (2017) 1498–1506.
[8] J. Hirschfeld, E.M. Akinoglu, D.C. Wirtz, A. Hoerauf, I. Bekeredjian-Ding, S. Jepsen, E.-M. Haddouti, A. Limmer, M. Giersig, Long-term release of antibiotics by carbon nanotube-coated titanium alloy surfaces diminish biofilm formation by Staphylococcus epidermidis, Nanomedicine: Nanotechnology, Biol. Med. 13 (4) (2017) 1587–1593.
[9] S. Amin Yavari, L. Loozen, F.L. Paganelli, S. Bakhshandeh, K. Lietaert, J.A. Groot, A.C. Fluit, C. Boel, J. Alblas, H.C. Vogely, Antibacterial behavior of additively manufactured porous titanium with nanotubular surfaces releasing silver ions, ACS Appl. Mater. Interfaces 8 (27) (2016) 17080–17089.
[10] I.A. van Hengel, M. Riool, L.E. Fratila-Apachitei, J. Witte-Bouma, E. Farrell, A.A. Zadpoor, S.A. Zaat, I. Apachitei, Selective laser melting porous metallic implants with immobilized silver nanoparticles kill and prevent biofilm formation by methicillin-resistant Staphylococcus aureus, Biomaterials 140 (2017) 1–15.
[11] Z. Jia, P. Xiu, P. Xiong, W. Zhou, Y. Cheng, S. Wei, Y. Zheng, T. Xi, H. Cai, Z. Liu, Additively manufactured macroporous titanium with silver-releasing micro-/nanoporous surface for multipurpose infection control and bone repair–a proof of concept, ACS Appl. Mater. Interfaces 8 (42) (2016) 28495–28510.
[12] B.S. Necula, L.E. Fratila-Apachitei, S.A. Zaat, I. Apachitei, J. Duszczyk, In vitro antibacterial activity of porous TiO2–Ag composite layers against methicillin-resistant Staphylococcus aureus, Acta Biomater. 5 (9) (2009) 3573–3580.
[13] B. Necula, J. Van Leeuwen, L. Fratila-Apachitei, S. Zaat, I. Apachitei, J. Duszczyk, In vitro cytotoxicity evaluation of porous TiO2–Ag antibacterial coatings for human fetal osteoblasts, Acta Biomater. 8 (11) (2012) 4191–4197.
[14] R.P. Singh, P. Ramarao, Cellular uptake, intracellular trafficking and cytotoxicity of silver nanoparticles, Toxicol. Lett. 213 (2) (2012) 249–259.
[15] L.Q. Chen, L. Fang, J. Ling, C.Z. Ding, B. Kang, C.Z. Huang, Nanotoxicity of silver nanoparticles to red blood cells: size dependent adsorption, uptake, and hemolytic activity, Chem. Res. Toxicol. 28 (3) (2015) 501–509.
[16] A. Gallardo-Godoy, C. Muldoon, B. Becker, A.G. Elliott, L.H. Lash, J.X. Huang, M. S. Butler, R. Pelingon, A.M. Kavanagh, S. Ramu, Activity and predicted nephrotoxicity of synthetic antibiotics based on polymyxin B, J. Med. Chem. 59 (3) (2016) 1068–1077.
[17] A. Elbourne, R.J. Crawford, E.P. Ivanova, Nano-structured antimicrobial surfaces: From nature to synthetic analogues, J. Colloid Interface Sci. 508 (2017) 603–616.
[18] A. Panáček, L. Kvítek, M. Smékalová, R. Večeřová, M. Kolář, M. Röderová, F. Dyčka, M. Šebela, R. Prucek, O. Tomanec, Bacterial resistance to silver nanoparticles and how to overcome it, Nat. Nanotechnol. 13 (1) (2018) 65.
[19] F. Song, M.E. Brasch, H. Wang, J.H. Henderson, K. Sauer, D. Ren, How bacteria respond to material stiffness during attachment: a role of Escherichia coli flagellar motility, ACS Appl. Mater. Interfaces 9 (27) (2017) 22176–22184.
[20] C. Díaz, M.C. Cortizo, P.L. Schilardi, S.G.G.d. Saravia, M.A.F.L.d. Mele, Influence of the nano-micro structure of the surface on bacterial adhesion, Mater. Res. 10 (1) (2007) 11–14.
[21] R.J. Crawford, H.K. Webb, V.K. Truong, J. Hasan, E.P. Ivanova, Surface topographical factors influencing bacterial attachment, Adv. Colloid Interface Sci. 179 (2012) 142–149.
[22] H. Gu, A. Chen, X. Song, M.E. Brasch, J.H. Henderson, D. Ren, How Escherichia coli lands and forms cell clusters on a surface: A new role of surface topography, Sci. Rep. 6 (2016) 29516.
[23] D.E. Discher, P. Janmey, Y.-L. Wang, Tissue cells feel and respond to the stiffness of their substrate, Science 310 (5751) (2005) 1139–1143.
[24] M.M. Stevens, J.H. George, Exploring and engineering the cell surface interface, Science 310 (5751) (2005) 1135–1138.
[25] M.J. Dalby, N. Gadegaard, R.O. Oreffo, Harnessing nanotopography and integrin–matrix interactions to influence stem cell fate, Nat. Mater. 13 (6) (2014) 558.
[26] S. Oh, K.S. Brammer, Y.J. Li, D. Teng, A.J. Engler, S. Chien, S. Jin, Stem cell fate dictated solely by altered nanotube dimension, Proc. Natl. Acad. Sci. 106 (7) (2009) 2130–2135.
[27] W.L. Murphy, T.C. McDevitt, A.J. Engler, Materials as stem cell regulators, Nat. Mater. 13 (6) (2014) 547.
[28] S. Dobbenga, L.E. Fratila-Apachitei, A.A. Zadpoor, Nanopattern-induced osteogenic differentiation of stem cells–a systematic review, Acta Biomater. 46 (2016) 3–14.
[29] K. Anselme, P. Davidson, A. Popa, M. Giazzon, M. Liley, L. Ploux, The interaction of cells and bacteria with surfaces structured at the nanometre scale, Acta Biomater. 6 (10) (2010) 3824–3846.
[30] K.K. Chung, J.F. Schumacher, E.M. Sampson, R.A. Burne, P.J. Antonelli, A.B. Brennan, Impact of engineered surface microtopography on biofilm formation of Staphylococcus aureus, Biointerphases 2 (2) (2007) 89–94.
[31] S. Janbaz, N. Noordzij, D.S. Widyaratih, C.W. Hagen, L.E. Fratila-Apachitei, A.A. Zadpoor, Origami lattices with free-form surface ornaments, Sci. Adv. 3 (11) (2017) eaao1595.
[32] A. Tripathy, P. Sen, B. Su, W.H. Briscoe, Natural and bioinspired nanostructured bactericidal surfaces, Adv. Colloid Interface Sci. 248 (2017) 85–104.
[33] B.D. Gates, Q. Xu, M. Stewart, D. Ryan, C.G. Willson, G.M. Whitesides, New approaches to nanofabrication: molding, printing, and other techniques, Chem. Rev. 105 (4) (2005) 1171–1196.
[34] Y. Sun, C.S. Chen, J. Fu, Forcing stem cells to behave: a biophysical perspective of the cellular microenvironment, Annu. Rev. Biophys. 41 (2012) 519–542.
[35] D.P. Linklater, S. Juodkazis, S. Rubanov, E.P. Ivanova, Comment on "Bactericidal Effects of Natural Nanotopography of Dragonfly Wing on Escherichia coli", ACS Appl. Mater. Interfaces 9 (35) (2017) 29387–29393.
[36] S. Ghosh, S. Niu, M. Yankova, M. Mecklenburg, S.M. King, J. Ravichandran, R.K. Kalia, A. Nakano, P. Vashishta, P. Setlow, Analysis of killing of growing cells and dormant and germinated spores of Bacillus species by black silicon nanopillars, Sci. Rep. 7 (1) (2017) 17768.
[37] I. Izquierdo-Barba, J.M. García-Martín, R. Álvarez, A. Palmero, J. Esteban, C. Pérez-Jorge, D. Arcos, M. Vallet-Regí, Nanocolumnar coatings with selective behavior towards osteoblast and Staphylococcus aureus proliferation, Acta Biomater. 15 (2015) 20–28.
[38] L. Feng, S. Li, Y. Li, H. Li, L. Zhang, J. Zhai, Y. Song, B. Liu, L. Jiang, D. Zhu, Super-hydrophobic surfaces: from natural to artificial, Adv. Mater. 14 (24) (2002) 1857–1860.
[39] K. Liu, L. Jiang, Bio-inspired design of multiscale structures for function integration, Nano Today 6 (2) (2011) 155–175.
[40] K. Modaresifar, S. Azizian, A. Hadjizadeh, Nano/biomimetic tissue adhesives development: from research to clinical application, Polym. Rev. 56 (2) (2016) 329–361.
[41] J. Hasan, H.K. Webb, V.K. Truong, S. Pogodin, V.A. Baulin, G.S. Watson, J.A. Watson, R.J. Crawford, E.P. Ivanova, Selective bactericidal activity of nanopatterned superhydrophobic cicada Psaltoda claripennis wing surfaces, Appl. Microbiol. Biotechnol. 97 (20) (2013) 9257–9262.
[42] M.N. Dickson, E.I. Liang, L.A. Rodriguez, N. Vollereaux, A.F. Yee, Nanopatterned polymer surfaces with bactericidal properties, Biointerphases 10 (2) (2015) 021010.
[43] G.S. Watson, D.W. Green, L. Schwarzkopf, X. Li, B.W. Cribb, S. Myhra, J.A. Watson, A gecko skin micro/nano structure–A low adhesion, superhydrophobic, anti-wetting, self-cleaning, biocompatible, antibacterial surface, Acta Biomater. 21 (2015) 109–122.

[44] F. Viela, I. Navarro-Baena, J.J. Hernández, M.R. Osorio, I. Rodriguez, Moth eye mimetic cytocompatible bactericidal nanotopography: A convergent design, Bioinspiration Biomimetics (2018).
[45] V.T. Pham, V.K. Truong, A. Orlowska, S. Ghanaati, M. Barbeck, P. Booms, A.J. Fulcher, C.M. Bhadra, R. Buividas, V. Baulin, "Race for the surface": eukaryotic cells can win, ACS Appl. Mater. Interfaces 8 (34) (2016) 22025–22031.
[46] J. Hasan, S. Raj, L. Yadav, K. Chatterjee, Engineering a nanostructured "super surface" with superhydrophobic and superkilling properties, RSC Adv. 5 (56) (2015) 44953–44959.
[47] P. Stiefel, S. Schmidt-Emrich, K. Maniura-Weber, Q. Ren, Critical aspects of using bacterial cell viability assays with the fluorophores SYTO9 and propidium iodide, BMC Microbiol. 15 (1) (2015) 36.
[48] J. Sjollema, S.A. Zaat, V. Fontaine, M. Ramstedt, R. Luginbuehl, K. Thevissen, J. Li, H.C. van der Mei, H.J. Busscher, In vitro methods for the evaluation of antimicrobial surface designs, Acta Biomater. 70 (2018) 12–24.
[49] Q. Yu, Z. Wu, H. Chen, Dual-function antibacterial surfaces for biomedical applications, Acta Biomater. 16 (2015) 1–13.
[50] H.J. Busscher, H.C. Van Der Mei, G. Subbiahdoss, P.C. Jutte, J.J. Van Den Dungen, S.A. Zaat, M.J. Schultz, D.W. Grainger, Biomaterial-associated infection: locating the finish line in the race for the surface, Sci. Transl. Med. 4(153) (2012) 153rv10-153rv10
[51] A. Persat, Bacterial mechanotransduction, Curr. Opin. Microbiol. 36 (2017) 1–6.
[52] C.D. Bandara, S. Singh, I.O. Afara, A. Wolff, T. Tesfamichael, K. Ostrikov, A. Oloyede, Bactericidal effects of natural nanotopography of dragonfly wing on Escherichia coli, ACS Appl. Mater. Interfaces 9 (8) (2017) 6746–6760.
[53] P. May, M. Clegg, T. Silva, H. Zanin, O. Fatibello-Filho, V. Celorrio, D. Fermin, C. Welch, G. Hazell, L. Fisher, Diamond-coated 'black silicon'as a promising material for high-surface-area electrochemical electrodes and antibacterial surfaces, J. Mater. Chem. B 4 (34) (2016) 5737–5746.
[54] E. Vassallo, M. Pedroni, T. Silvetti, S. Morandi, S. Toffolatti, G. Angella, M. Brasca, Bactericidal performance of nanostructured surfaces by fluorocarbon plasma, Mater. Sci. Eng. C (2017).
[55] V.K. Truong, N.M. Geeganagamage, V.A. Baulin, J. Vongsvivut, M.J. Tobin, P. Luque, R.J. Crawford, E.P. Ivanova, The susceptibility of Staphylococcus aureus CIP 65.8 and Pseudomonas aeruginosa ATCC 9721 cells to the bactericidal action of nanostructured Calopteryx haemorrhoidalis damselfly wing surfaces, Appl. Microbiol. Biotechnol. 101 (11) (2017) 4683–4690.
[56] Y. Cao, B. Su, S. Chinnaraj, S. Jana, L. Bowen, S. Charlton, P. Duan, N.S. Jakubovics, J. Chen, Nanostructured titanium surfaces exhibit recalcitrance towards Staphylococcus epidermidis biofilm formation, Sci. Rep. 8 (1) (2018) 1071.
[57] S.M. Kelleher, O. Habimana, J. Lawler, B. O'Reilly, S. Daniels, E. Casey, A. Cowley, Cicada wing surface topography: an investigation into the bactericidal properties of nanostructural features, ACS Appl. Mater. Interfaces 8 (24) (2015) 14966–14974.
[58] E.P. Ivanova, J. Hasan, H.K. Webb, G. Gervinskas, S. Juodkazis, V.K. Truong, A.H. Wu, R.N. Lamb, V.A. Baulin, G.S. Watson, Bactericidal activity of black silicon, Nat. Commun. 4 (2013).
[59] G. Yi, Y. Yuan, X. Li, Y. Zhang, ZnO Nanopillar Coated Surfaces with Substrate-Dependent Superbactericidal Property, Small 14 (14) (2018) 1703159.
[60] G. Jin, H. Qin, H. Cao, S. Qian, Y. Zhao, X. Peng, X. Zhang, X. Liu, P.K. Chu, Synergistic effects of dual Zn/Ag ion implantation in osteogenic activity and antibacterial ability of titanium, Biomaterials 35 (27) (2014) 7699–7713.
[61] W. Wang, T.L. Li, H.M. Wong, P.K. Chu, R.Y. Kao, S. Wu, F.K. Leung, T.M. Wong, M.K. To, K.M. Cheung, Development of novel implants with self-antibacterial performance through in-situ growth of 1D ZnO nanowire, Colloids Surf. B 141 (2016) 623–633.
[62] K. Nowlin, A. Boseman, A. Covell, D. LaJeunesse, Adhesion-dependent rupturing of Saccharomyces cerevisiae on biological antimicrobial nanostructured surfaces, J. R. Soc. Interface 12 (102) (2015) 20140999.
[63] S.L. Walker, J.E. Hill, J.A. Redman, M. Elimelech, Influence of growth phase on adhesion kinetics of Escherichia coli D21g, Appl. Environ. Microbiol. 71 (6) (2005) 3093–3099.
[64] Z. Xu, Y. Lai, D. Wu, W. Huang, S. Huang, L. Zhou, J. Chen, Increased Mesenchymal stem cell response and decreased Staphylococcus aureus adhesion on titania nanotubes without pharmaceuticals, Biomed Res. Int. 2015 (2015).
[65] S. Aguayo, A. Strange, N. Gadegaard, M. Dalby, L. Bozec, Influence of biomaterial nanotopography on the adhesive and elastic properties of Staphylococcus aureus cells, RSC Adv. 6 (92) (2016) 89347–89355.
[66] F. Hizal, C.-H. Choi, H.J. Busscher, H.C. van der Mei, Staphylococcal adhesion, detachment and transmission on nanopillared si surfaces, ACS Appl. Mater. Interfaces 8 (44) (2016) 30430–30439.
[67] L.E. Fisher, Y. Yang, M.-F. Yuen, W. Zhang, A.H. Nobbs, B. Su, Bactericidal activity of biomimetic diamond nanocone surfaces, Biointerphases 11 (1) (2016) 011014.
[68] D.P. Linklater, H.K.D. Nguyen, C.M. Bhadra, S. Juodkazis, E.P. Ivanova, Influence of nanoscale topology on bactericidal efficiency of black silicon surfaces, Nanotechnology 28 (24) (2017) 245301.
[69] S. Wu, F. Zuber, K. Maniura-Weber, J. Brugger, Q. Ren, Nanostructured surface topographies have an effect on bactericidal activity, J. Nanobiotechnol. 16 (1) (2018) 20.
[70] S. Pogodin, J. Hasan, V.A. Baulin, H.K. Webb, V.K. Truong, V. Boshkovikj, C.J. Fluke, G.S. Watson, J.A. Watson, R.J. Crawford, Biophysical model of bacterial cell interactions with nanopatterned cicada wing surfaces, Biophys. J. 104 (4) (2013) 835–840.
[71] T. Diu, N. Faruqui, T. Sjöström, B. Lamarre, H.F. Jenkinson, B. Su, M.G. Ryadnov, Cicada-inspired cell-instructive nanopatterned arrays, Sci. Rep. 4 (2014).
[72] E. Fadeeva, V.K. Truong, M. Stiesch, B.N. Chichkov, R.J. Crawford, J. Wang, E.P. Ivanova, Bacterial retention on superhydrophobic titanium surfaces fabricated by femtosecond laser ablation, Langmuir 27 (6) (2011) 3012–3019.
[73] X. Li, G. Cheung, G.S. Watson, J.A. Watson, S. Lin, L. Schwarzkopf, D. Green, The nanotipped hairs of gecko skin and biotemplated replicas impair and/or kill pathogenic bacteria with high efficiency, Nanoscale 8 (45) (2016) 18860–18869.
[74] M.G. Mazza, The physics of biofilms—an introduction, J. Phys. D Appl. Phys. 49 (20) (2016) 203001.
[75] D.H. Limoli, C.J. Jones, D.J. Wozniak, Bacterial extracellular polysaccharides in biofilm formation and function, Microbiol. Spectrum 3 (3) (2015).
[76] K.M. Colvin, V.D. Gordon, K. Murakami, B.R. Borlee, D.J. Wozniak, G.C. Wong, M. R. Parsek, The pel polysaccharide can serve a structural and protective role in the biofilm matrix of Pseudomonas aeruginosa, PLoS Pathog. 7 (1) (2011) e1001264.
[77] A. Dragoš, Á.T. Kovács, The peculiar functions of the bacterial extracellular matrix, Trends Microbiol. 25 (4) (2017) 257–266.
[78] B.J. Cooley, T.W. Thatcher, S.M. Hashmi, G. L'Her, H.H. Le, D.A. Hurwitz, D. Provenzano, A. Touhami, V.D. Gordon, The extracellular polysaccharide Pel makes the attachment of P. aeruginosa to surfaces symmetric and short-ranged, Soft Matter 9 (14) (2013) 3871–3876.
[79] L. Rizzello, B. Sorce, S. Sabella, G. Vecchio, A. Galeone, V. Brunetti, R. Cingolani, P.P. Pompa, Impact of nanoscale topography on genomics and proteomics of adherent bacteria, ACS Nano 5 (3) (2011) 1865–1876.
[80] E.K. Yim, E.M. Darling, K. Kulangara, F. Guilak, K.W. Leong, Nanotopography-induced changes in focal adhesions, cytoskeletal organization, and mechanical properties of human mesenchymal stem cells, Biomaterials 31 (6) (2010) 1299–1306.
[81] T. Sjöström, L.E. McNamara, R.D. Meek, M.J. Dalby, B. Su, 2D and 3D nanopatterning of titanium for enhancing osteoinduction of stem cells at implant surfaces, Adv. Healthcare Mater. 2 (9) (2013) 1285–1293.
[82] L.E. McNamara, T. Sjöström, K.E. Burgess, J.J. Kim, E. Liu, S. Gordonov, P.V. Moghe, R.D. Meek, R.O. Oreffo, B. Su, Skeletal stem cell physiology on functionally distinct titania nanotopographies, Biomaterials 32 (30) (2011) 7403–7410.
[83] S. Guvendik, L. Trabzon, M. Ramazanoglu, The effect of Si nano-columns in 2-D and 3-D on cellular behaviour: nanotopography-induced CaP deposition from differentiating mesenchymal stem cells, J. Nanosci. Nanotechnol. 11 (10) (2011) 8896–8902.
[84] R.K. Silverwood, P.G. Fairhurst, T. Sjöström, F. Welsh, Y. Sun, G. Li, B. Yu, P.S. Young, B. Su, R.M. Meek, Analysis of osteoclastogenesis/osteoblastogenesis on nanotopographical titania surfaces, Adv. Healthcare Mater. 5 (8) (2016) 947–955.