Nanotechnology for Reducing Orthopedic Implant Infections: Synthesis, Characterization, and Properties

Luting Liu and Thomas J. Webster

Keywords Bone · Bacteria · Microorganisms · Nanotextured · Topography · Surface roughness · Nanoparticles · Infection · Nanomedicine · Nano-textured · Morphology · Nanometer

1 Introduction

1.1 Current Implants and Implantable Devices

Each year millions of patients improve their quality of life through surgical procedures that involve implants or implantable medical devices. Medical implants are devices or tissues that are placed inside or on the surface of the body. Many implants are prosthetics, intended to replace or restore the function of traumatized or degenerated tissues and organs. Other implants deliver medication, monitor body functions, or provide support to organs and tissues [1]. Currently, implants are being used in many different parts of the body for various applications such as orthopedics, pacemakers, cardiovascular stents, and catheters [2]. Concurrent with the increased life span in today's world, the number of age-related diseases has also increased. For example, the global orthopedic implants market was valued at USD 4.3 billion in 2015 and is expected to reach USD 6.2 billion by 2024, according to a new report by Grand View Research, Inc. The constantly rising geriatric population is primarily driving the growth of the market since people aged above 65 years are at a high risk of developing degenerative disc disease, low bone density, and osteoarthritis [3].

In addition, the increasing number of sports-related injuries along with the growing phenomena of road accidents is fueling the number of trauma cases, thereby propelling the demand for orthopedic implants. Cardiovascular diseases are another example. Over the last two decades, coronary stents have become a new standard in angioplasty procedures [4]. In 2004, the number of implanted drug-eluting stents

L. Liu · T. J. Webster (✉)
Department of Chemical Engineering, Northeastern University, Boston, MA, USA
e-mail: th.webster@neu.edu

B. Li, T. Webster (eds.), *Orthopedic Biomaterials*,
https://doi.org/10.1007/978-3-319-73664-8_2

[2] alone exceeded two million. Increasing incidence rates of cardiovascular diseases favors the growth of cardiovascular interventional procedures. The global cardiovascular implants market is poised to grow at a CAGR of around 4.5% over the next decade [5]. In 2014, the global catheters market size was estimated at USD 26.38 billion, of which cardiovascular catheters accounted for the largest product segment at USD 10.17 billion, and is expected to grow at a CAGR of 9.7% from 2014 to 2020 [6]. The rising demand for implants and implantable medical devices across the globe leads to a significant rise in demand for biomaterials [7]. Transparency Market Research estimates that the global biomaterials' market will exhibit a healthy 4.1% CAGR from 2013 and 2019, rising to a valuation of US $33,600 million by 2019. The most important criterion for the long-term success of implants is the selection of a suitable implant biomaterial.

To improve the biological performance of an implant, it is necessary to select a material that does not elicit any negative biological responses and at the same time maintains adequate function [8, 9]. It is mandatory for the scientists, engineers and clinicians to have a comprehensive knowledge of various biomaterials used for implants. However, this is not enough. For the future of successful implants, we must think beyond simply creating materials that do not create a negative response, but one that understands the patient's reaction to the implant and responds in real time to correct any negative reaction. This is because, as has been proven, there are several different responses patient's may have to the same implant chemistry due to altered immune systems. Thus, implants of the future must be intelligent to first recognize tissue/cell responses and secondly respond accordingly.

2 Implant Biomaterials

Biomaterials are chiefly sourced or synthesized from a variety of metals, polymers, and ceramics. Metals are based on metallic bonds, ceramics are based on ionic bonds, and polymers are based on covalent bonds. In terms of the type of biomaterials used in implantable devices, metals are currently the most preferred materials [10]. The segment of metals accounted for a more than 50% of the global market's revenue in 2012 and is expected to remain the dominant contributor of revenue to the global market in the next few years as well [7]. The segment of polymers, which currently holds the second position in terms of share in the market, however, is expected to exhibit the most promising growth rate over the forecasted period. The vast advances in polymer technologies, easy availability of a number of biocompatible polymers, and continuous research and development activities in the field of biopolymers are expected to encourage implantable device manufacturers to consider the increased use of polymers. Polymers also have an upper hand over metals for medical applications, especially when it comes to elasticity, flexibility, longevity, and bio-inertness [11].

Conventionally, as mentioned, the best performance of the vast majority of implantable devices is achieved when the biomaterials used in their construction are

chemically and biologically inert; no biological, let alone pharmacological, activity used to be sought in these devices. However, today, at least in theory, there are numerous exceptions, where it now seems that inert medical devices are not enough to get past our currently unacceptable high rate of failure. For example, we now need materials that proactively promote biological activity (such as bone regeneration) or minimize undesirable activity (such as infection or blood clotting) [12–14]. The sections below highlight current advances for traditional categories of orthopedic implants.

2.1 Metals

As a class of materials, metals are most widely used for load-bearing implants. Due to their high mechanical strength, metallic materials were utilized in orthopedic applications as early as the 1890s. Although many metals and alloys are used for medical device applications, the most commonly employed are stainless steels, cobalt-chromium alloys, commercially pure titanium, and its alloys [10, 15]. Various properties of these metallic implant materials are listed in Table 1.

Among metals used in orthopedics, stainless steels exhibit a moderate to high elastic modulus and tensile strength. Additionally, these steels possess good ductility, which allows them to be cold worked [16]. Compared to stainless steel, cobalt-chromium alloys exhibit higher elastic modulus, strength and hardness, but they have relatively low ductility and are difficult to machine. Cobalt-chromium alloys are highly corrosion resistant. They possess adequate fatigue properties to serve as artificial joints or total joint prostheses and are used extensively for this purpose [17].

Titanium (Ti) and titanium alloys are relatively new materials compared with stainless steels and cobalt-chromium alloys [5]. They are well known for their

Table 1 Comparison of some of the characteristics and properties of metallic implant materials

	Stainless steels	Cobalt-chromium alloys	Ti and Ti alloys
Young's modulus (GPa)	200	230	106
Tensile strength (MPa)	540–1000	900–1540	900
Advantages	Cost; Availability; Good ductility; Processing	Wear resistance; Corrosion resistance; Fatigue strength	Biocompatibility; Corrosion resistance; Minimum modulus; Fatigue strength
Disadvantages	Long-term behavior; High modulus	High modulus; Biocompatibility	Low wear resistance; Low shear strength:
Applications	Temporary devices (fracture plates, screws, hip nails) for hip replacement	Dentistry castings; Prostheses stems; Load-bearing components in joint replacement	Long-term permanent devices (nails, pacemakers); Intraosseous-dental implants

excellent corrosion resistance and high specific strength. The main drawback of Ti and its alloys is their inadequate wear resistance [18, 19]. In consideration of the biocompatibility of the metals and alloys, the susceptibility of the material to corrosion and the effect the corrosion has on the tissue are the key aspects [20, 21]. Corrosion resistance of the currently used 316L stainless steel, cobalt-chromium, and Ti-based implant alloys relies on their passivation by a thin surface layer of oxide. Stainless steel is one of the least corrosion resistant metals and never appears to fully integrate with bone or soft tissue, thus, it is usually used for temporary implants only. Ti and cobalt-chromium alloys do not corrode in the body; however, metal ions slowly diffuse through the oxide layer and accumulate in the tissue.

When a metal implant is placed in the human body, it becomes surrounded by a layer of fibrous tissue of a thickness that is proportional to the amount and toxicity of the dissolution products and to the amount of motion between the implant and the adjacent tissues [22]. The proliferation of a fibrous layer as much as 2 mm thick is encountered with the use of stainless steel implants, while Ti may elicit a minimal fibrous encapsulation under some conditions comparatively. Ti was found to be the only metallic biomaterial to osseointegrate presumably due to the slow growth of hydrated titanium oxide on the surface of the Ti implant that leads to the incorporation of calcium and phosphorus [23]. In general, metals are by far the oldest materials for fabricating implantable devices. They are still the most preferred materials currently, and will continue to dominate the market in the next few years as well [6]. However, as produced, the metals mentioned above do not have any ability to inhibit or slow the growth of bacteria that leads to infection.

2.2 Polymers

Polymeric materials are rapidly replacing other material classes such as metals and ceramics for use as biomaterials because of their versatility. Their applications range from facial prostheses, endotracheal tubes to dentures, hip and knee joints. Various synthetic and natural polymers are used in such implants and devices [11, 24]. Many researchers consider natural polymers to have additional benefits over synthetic polymers, such as their biodegradable properties. However, synthetic polymers have been the material of choice for implants because of their ease of production, availability and versatility of manipulation [25]. There are many other types of commercially available synthetic polymers used in implants and devices, which are listed in Table 2.

As the polymer molecular weight increases, material strength also increases while elasticity decreases. Ultrahigh molecular weight polyethylene (UHMWPE) was the first polymeric material used in medicine since the 1960s. UHMWPE is highly resistant to corrosive chemicals and has extremely low moisture absorption, a very low coefficient of friction, characteristic of self-lubrication and high resistance to abrasion. UHMWPE emerged as a bearing material in many joint replacement devices [26].

Table 2 Polymers used for implantable devices

Polymers	Applications
Polyethylene (PE)	Joint replacement devices, total hip arthroplasty
Polypropylene (PP)	Heart valve structures, surgical mesh, sutures
Polymethylmethacrylate (PMMA)	Dental restorations, intraocular lenses, bone cements in total joint replacements
Polyetheretherketone (PEEK)	Partial replacement of skull
Polyethylene terephthalate (PET)	Vascular grafts and prosthesis, shunt, sutures
Silicones\polydimethylsiloxane (PDMS)	Encapsulate material in implants, catheters, tubing, shunt, packaging materials for implantable devices
Polytetrafluoroethylene (PTTE)	Catheter coating, vascular graft, vascular prostheses
Polyamides (Nylons)	Sutures
Polyurethane (PU)	Breast implants, catheter coatings
Polyvinylchloride (PVC)	Tubing, catheters, blood containers

Polypropylene (PP), similarly to PE, is a thermoplastic polymer that can also be altered according to its density. PP has been widely used as surgical mesh to reinforce weakened tissues while also acting as a scaffold for fibro-collagenous tissues to grow on the mesh itself and has mainly been applied in urogynecology to treat stress urinary incontinence and pelvic organ prolapse. Recently, numerous studies have examined its use in other parts of the body, such as for implant-based breast reconstruction [27]. PP has also been used together with titanium to produce a mesh with a thinner capsular contracture, which is a major complication in implant-based breast reconstruction. It is also a good material that can be used as a supportive soft tissue structure [28].

Polymethylmethacrylate (PMMA) has been used in various medical implants such as in intraocular lenses, dental implant restoration, and as bone cements in total joint replacements [29–31]. The primary purpose of PMMA as a bone cement is to fill the space between the prostheses and bone to achieve a more uniform stress distribution, and bone cements do not serve as adhesives [32]. However, PMMA does not support osseointegration [8], which restricts its applicability to a great degree. PMMA was the first material to be successfully used in intraocular lenses in the eye when the original lens is removed in the treatment of cataracts or myopia [33]. Tissue growth for PMMA orbital implants has also been tested, and results showed that fibro-vascular ingrowth of tissues from surrounding orbital tissues in the eyes could be achieved with no signs of infection. Intraocular lenses have also been developed using PMMA, and the results showed that the chromatic difference of focus values were similar to the physiological values measured in young eyes.

Polyetheretherketone (PEEK), a thermoplastic polymer, approved as a medical grade material by the U.S. FDA in the late 1990s, has recently been studied and used as a substitute for metallic implant materials because of its appropriate biocompatibility and extremely low elastic modulus (3–4 GPa), which reduces the extent of stress shielding that is often observed in Ti-based metallic implants [34, 35]. PEEK is also considered as an advanced biomaterial used with a high-resolution magnetic

resonance imaging (MRI), for creating a partial replacement of the skull in neurosurgical applications. The use of polyethylene terephthalate (PET) in medical devices has endured for more than 50 years. Current medical applications of PET include implantable sutures, surgical mesh, vascular grafts, heart valve sewing cuffs and components for percutaneous access devices due to its notable biological characteristics of biostability and promotion of tissue ingrowth [9, 36].

Silicones are polymers that include any inert, synthetic compound made up of repeating units of siloxane, have been widely used as an encapsulant material in implants (i.e., breast implants, testicle implants, pectoral implants) [37]. Silicone was studied to be the most reliable for long-term encapsulation in the body compared to epoxy resin and polyurethane because of their lower surface energy and smoother topography [38]. These features also prevent cells and molecules from being absorbed by the polymer itself. There were also fewer defects observed on the silicon surface, indicating better protective functions. Polydimethylsiloxane (PDMS) is a common derivative of silicone that has been used in pacemakers, blood pumps, mammary prostheses, catheters, shunts, cochlear implants, esophagus replacements, and as a packaging material for implantable electronic devices and sensors [39]. Polytetrafluoroethylene (PTFE) (also called Teflon) was developed by DuPont Co, which is frequently employed as a coating on catheters. PTFE coated catheters are also commonly used to drain urine after surgeries and have recently been used as controls in further research to reduce infections. Expanded PTFE grafts are clinically acceptable for peripheral vascular surgery and arteriovenous shunts [36, 40].

Polyamide (PA) is a macromolecule with repeating units linked by amide bonds. The most common form of PA used in biomedical implants and devices is nylon, which is often used as a material for fibers in composites to increase the mechanical strength of the composite, such as suture materials. Nylon has been tested to study microbial [10] contamination and results indicated that nylon has the ability to prevent bacterial transmission [41]. Polyurethane (PU) has been used in a wide range of implants and can also be easily modified to fit different biomedical applications. However, PU can be affected by chemical attacks in vivo, resulting in the degradation of the material. When handled correctly, the degradation kinetics can be controlled to facilitate the growth of new tissues. It was also found that PU has a lower water permeability and PU breast implants showed very low rates of capsular contracture [42, 43].

Polyvinylchloride (PVC) is the world's third-most widely produced synthetic plastic polymer, after PE and PP. The two main applications areas for medically approved PVC compounds are blood containers and tubing. In Europe, the consumption of PVC for medical devices is approximately 85,000 tons every year. The reasons for using flexible PVC in these applications for over 50 years are numerous and based on cost effectiveness linked to transparency, light weight, softness, tear strength, kink resistance, suitability for sterilization and biocompatibility [25]. Potentially, devices made from bioresorbable polymers can overcome problems associated with metal implants like stress protection, potential for corrosion, wear and debris formation as well as the necessity of implant removal. The most

commonly investigated and widely used synthetic biodegradable polymers are polyglycolide (PGA), polylactide (PLA) and polycaprolactone (PCL) [44, 45]. These polymers are well known for their good biocompatibility, with their degradation products being eliminated from the body by metabolic pathways. Many reports have shown that the different PLA-based substrates do not present toxicity since the cells were found to differentiate over the different polymers, as demonstrated by the production of extracellular matrix components by various cell types [46].

However, as in the case of metals, none of these polymers mentioned above were developed to reduce infection and/or bacteria growth and do not possess such properties as used today.

2.3 Ceramics

Restorative materials in dentistry such as crowns, cements and dentures are composed of ceramic materials. The poor fracture toughness of ceramics severely limits their use for load-bearing applications. They are generally used to replace or fix hard connective tissue, such as bone [9, 47]. Bone itself is a composite, comprising an organic phase and an inorganic phase. This inorganic phase is mainly composed of crystalline calcium hydroxyapatite with the chemical formula $Ca_{10}(PO_4)_6(OH)_2$. Thus, synthetic calcium hydroxyapatite is a good candidate for a successful biomaterial. Several dental and orthopedic metal implants are coated with hydroxyapatite to ensure long-term fixation in bone [48]. Zirconium dioxide or zirconia ceramics, a bioinert nonresorbable metal oxide, are used to manufacture femoral heads for total hip replacements and are potentially suitable for the highly mechanically loaded environments found in joint replacements due to its high strength, toughness and surface finish [49]. A ceramic that is used in load-bearing applications is high-purity alumina. High purity alumina bioceramics have been developed as an alternative to surgical metal alloys for total hip prosthesis and tooth implants. The high hardness, low friction coefficient and excellent corrosion resistance of alumina offers a very low wear rate at the articulating surfaces in orthopedic applications. Most importantly, over 3000 alumina implants monitored by the FDA have been successfully implemented since 1987 [50].

Again, however, the ceramics mentioned above possess no inherent properties, nor were designed to, reduce infection and/or bacteria adhesion and growth.

3 Problems with Conventional Implants

The risks of implantable medical devices include surgical risks during placement or removal and implant failure due to mechanical (i.e., stress-strain imbalances, implant migration and wear debris) or biological (most notably, foreign body reactions and bacterial infections) factors [51–53]. Herein, biological factors will be the

primary focus, particularly infection due to their growing concern. To highlight this problem, the Centers for Disease Control recently predicted that deaths due to infection and antibiotic-resistant bacteria will overcome the number of people who die from all cancers by 2050; such alarming statistics highlight the need for more attention to design medical devices that inhibit bacteria functions.

3.1 Host Response to Foreign Materials

When an implant is inserted into the body, some immediate biological reactions will take place near the implanted area. This is essentially the body's response to a newly implanted foreign material [54]. The foreign body reaction is composed of macrophages and foreign body giant cells as the end-stage response of the inflammatory and wound healing responses following implantation of a medical device, prosthesis, or biomaterial. The chemical, physical, and morphological characteristics of the biomaterial surfaces are considered to play an important role in modulating the foreign body reaction in the first few weeks following implantation of a medical device, even though the foreign body reaction at the tissue/material interface is present for the entire in vivo lifetime of the medical device [54]. An understanding of the foreign body reaction is important as the foreign body reaction may impact the biocompatibility of the medical device, prosthesis, or implanted biomaterial and may directly determine the success or failure of implantation [13, 55]. After the implantation procedure, the body follows a sequence of local events during the healing response. In order, these are acute inflammation, chronic inflammation, granulation tissue formation, foreign body reaction and fibrosis. Inflammation is the reaction of vascularized living tissue to local injury. The inflammatory response comprises an initial acute phase and a subsequent chronic phase. The initial inflammatory response is activated regardless of the type of biomaterial and the location of injury [56, 57].

The acute phase lasts from hours to days and is marked by fluid and protein exudation as well as a neutrophilic reaction. Neutrophils are recruited to the site of inflammation by chemical mediators to phagocytize microorganisms and foreign materials. Afterwards, neutrophils recruit monocytes to the inflammation area (where monocytes will further differentiate into macrophages). They attach on the surfaces of the biomaterial by adsorbed proteins, basically immunoglobulin G (IgG) and complement-activated fragment (C3b) [58]. Proteins adsorb to an implanted material instantaneously after being inserted in the body. Macrophages then secrete degradative agents (such as superoxides and free radicals), which severely damage the implant. Persistency of the inflammatory response leads to chronic inflammation. Main cell types observed during chronic inflammation are monocytes, macrophages and lymphocytes. Macrophages are the most important type of cells in chronic inflammation due to the secretion of a great number of biologically active products such as: proteases, arachidonic acid metabolites, reactive oxygen

metabolites, coagulation factors and growth factors (which are important to recruit fibroblasts and epithelial cells) [14, 59].

The third step in the foreign body response is granulation tissue formation. Endothelial cells and fibroblasts form granulation tissue. This tissue is the hallmark of the healing response. It is granular in appearance and contains many small blood vessels. In addition, macrophages fuse together to form foreign body giant cells to phagocytize the foreign materials more effectively. The amount of granulation tissue determines the extent of fibrosis. The foreign body reaction, the fourth step in wound healing, contains foreign body giant cells and granulation tissue (such as fibroblasts, capillaries and macrophages). The last step in the wound healing response is fibrosis, which is the fibrous tissue encapsulation of the implant [60]. This fibrous wall confines the implant and consequently prevents it from interacting with the surrounding tissue [8].

The resulting collagenous fibrotic capsule, up to several hundred micrometers thick, physically and physiologically separates the device from host tissue. Lacking vasculature, the capsule can be impermeable to cells and can hinder metabolite transport, slow healing, resist device-tissue integration and create niches susceptible to infection [61]. Thus, outwitting the natural immune response is the most formidable challenge, which drives the demand for developing novel implant biomaterial surfaces to provoke a significant foreign-body response.

Of course, missing from this traditional explanation of the foreign body reaction to implants are bacteria. It is now well understood that some bacteria will be present in any surgical site due to the presence of bacteria on one's own skin (e.g., *Staph. epidermidis*). An on-going debate in the field is whether one wants an extensive inflammatory response to clear such bacteria from the implant surface, or to minimize the inflammatory response since chronic inflammation reduces bone (and/or any tissue) formation juxtaposed to the implant.

3.2 Bacterial and Biofilm Infections

The implant surface is susceptible to infection because of two main reasons, namely formation of a surface biofilm and compromised immune ability at the implant/tissue interface [61, 62]. Many patients are on immune suppressants after surgery which, while decreasing the chances for chronic inflammation, also increases the chances for infection. Infection has been reported on numerous implantable devices including central venous catheters, endotracheal tubes, intrauterine devices, orthopedic joint prosthetics, and percutaneous orthopedic devices, etc. [63]. A very large proportion of all implant-related infections are caused by staphylococci, specifically *Staphylococcus aureus* (*S. aureus*) and *Staphylococcus epidermidis* (*S. epidermidis*). Staphylococcus is a genus of Gram-positive bacteria, nonspore forming facultative anaerobes that grow by aerobic respiration or fermentation, with diameters of 0.5–1.5 μm. Staphylococcus comprises up to two-thirds of all pathogens in implant infections, which are difficult to treat due to the ability of the organisms to form

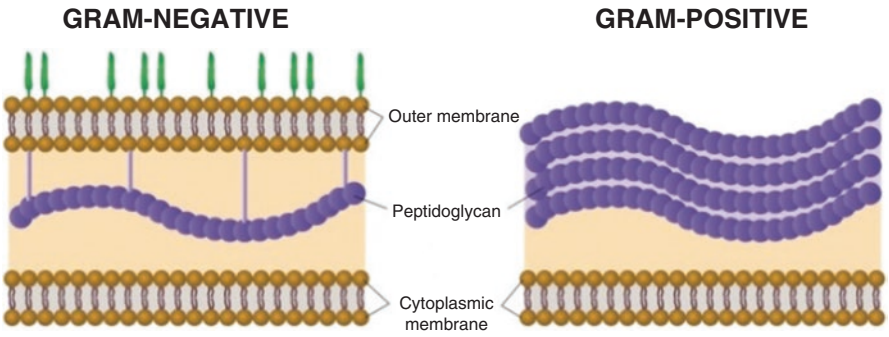

Fig. 1 The differences between the membrane structures of Gram-negative and Gram-positive bacteria. Due to the more structurally sound membrane of gram-negative bacteria, it is more difficult to kill gram-negative bacteria

small colonies and further to grow into biofilms [64]. Gram-negative bacteria, such as *Pseudomonas aeruginosa* (*P. aeruginosa*) and *Escherichia coli* (*E. coli*), account for 15% of the infections as well, whose presence can lead to infections such as urinary tract infections [65]. Gram-positive bacteria possess a thick cell wall containing many layers of peptidoglycan and teichoic acids. Gram-negative bacteria have thinner cell walls but are surrounded by a second lipid membrane containing a substance known as lipopolysaccharide. The lipopolysaccharide containing outer membrane of a Gram-negative bacterium results in a greater resilience to antibiotics and other antimicrobial agents than Gram-positive bacteria (Fig. 1) [66].

In addition, due to the widespread use of antibacterial therapy around 60 years ago, bacterial antibiotic resistance has rapidly increased due to their overuse. Antibiotic resistance amongst bacteria increases proportionally with antibiotic exposure as these resistant microorganisms have a greater chance of survival, reproduction and multiplication than their drug-sensitive counterparts. Infections caused by drug resistant bacteria, such as methicillin-resistant *S. aureus* (MRSA) and *E. coli*, have been acknowledged as a growing and significant problem in hospitals and healthcare facilities [67, 68]. Thus, it can be argued that the prevention of bacterial adhesion without drugs may be one of the best ways to reduce implant associated infections. Implant associated infections can be caused by the adhesion of bacteria on the implant surface followed by biofilm formation. Adhesion of bacteria to human tissue surfaces and implanted biomaterial surfaces is the first and most important step in the pathogenesis of infection, whereby the bacteria can divide and colonize the surface. Bacterial adhesion to a material surface can be described as a two-phase process including an initial, instantaneous and reversible physical phase (Phase I) and a time-dependent and irreversible molecular and cellular phase (Phase II) [69].

Bacterial adhesion to surfaces consists of the initial attraction of the cells to the surface followed by adsorption and attachment. Bacteria move to or are moved to a material surface through the effects of physical forces, such as Brownian motion, van der Waals attraction forces, gravitational forces, surface electrostatic charge and

hydrophobic interactions. These physical interactions are further classified as long-range and short-range interactions. The long-range interactions (non-specific, distances >50 nm) between cells and material surfaces are described by mutual forces, which are related to the distance and free energy. Short-range interactions become effective when the cell and the surface come into close contact (<5 nm), these can be separated into chemical bonds (such as hydrogen bonding), ionic and dipole interactions and hydrophobic interactions [70]. This initial attachment of bacteria to surfaces is the initial part of adhesion, which makes the molecular or cellular phase of adhesion possible.

In the Phase II of adhesion, molecular specific reactions [18] between bacterial surface structures and substratum surfaces become predominant. This implies a firmer adhesion of bacteria to a surface by the selective bridging function of bacterial surface polymeric structures, which include capsules, fimbriae or slime. In fact, the functional part of these structures should be the adhesins, especially when the substrata are host tissues [71]. Beyond Phase II, certain bacterial strains are capable of forming a biofilm if provided with an appropriate supply of nutrients, which could protect the microorganisms from the host immune system and antibiotic therapy [72]. It has been reported by the National Institutes of Health that 80% of all chronic infections are due to biofilms. A biofilm is an aggregate of bacteria in which bacterial cells adhere to each other on a wet or moist surface. Biofilms may form on living or non-living surfaces and can be prevalent in natural, industrial and hospital settings [73]. The formation of a biofilm (Fig. 2) begins with the attachment of free-floating bacteria to the surface. Along with the generation of exopolysaccharide, the attachment of bacteria becomes irreversible. As the bacteria propagate quickly, the biofilm structure develops and becomes more complicated. At the last stage of biofilm growth, the bacteria release into the environment and contaminate other surfaces [74]. Biofilms are considered easy to form but hard to treat, which can cause wide-spread infections in the human body; for example, through catheter infections, infections on inert surfaces of artificial implants, etc. [75]. These biofilm infections can be serious and hard to treat because the development of the biofilm structure may allow for bacteria to be increasingly antibiotic resistant, because the bacteria in the biofilm is held together and protected by a matrix of EPS (extracellular polymeric

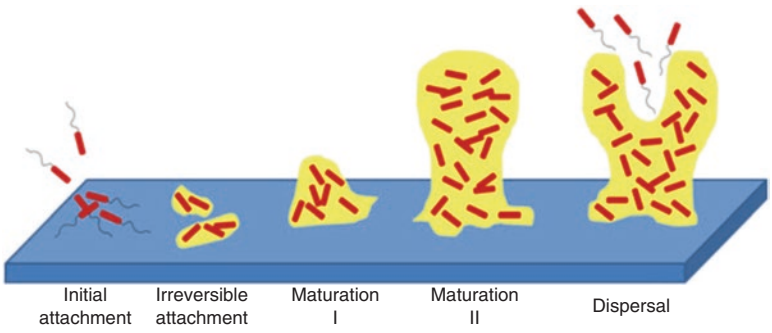

Fig. 2 Major stages of biofilm formation

substance or exopolysaccharide). This matrix protects bacteria cells within it and facilitates communication among them through biochemical signals, resulting in their increased resistance to detergents and antibiotics [76].

Bacterial adhesion and biofilm formation processes are influenced by environmental factors, bacterial properties, material surface properties and the presence of serum or tissue proteins. Properties of the substrate, such as chemical composition of the material, surface charge, hydrophobicity, surface roughness and the presence of specific proteins at the surface, are all thought to be important in the initial cell attachment process. Reduction of microbial adhesion to an implant (without the use of drugs) could be an attractive method for reducing infection and nanotechnology may hold some answers.

4 Where Bio Meet Nano: The Use of Nanotechnology in Implants

Implanted materials trigger an immune system response to isolate non-natural materials [20]. Once any foreign material enters the body, a natural inflammatory response immediately kicks in, coating the intruder's surface with thousands of proteins and marshaling immune cells such as macrophages to remove it, making the implant eventually blind to the biochemistry outside. Outwitting the natural immune response is the most formidable challenge in this field. All of these bring a large challenge of developing and inducing biocompatible implant materials. In order to accomplish this, we try to make foreign implants look natural in the body [77]. Nanotechnology (involved in designing and engineering of materials, devices and systems on the order of less than 100 nm) is one of the current fields of high interest that can control properties of implants and tissue engineering scaffolds. It deals with systems and structures that result in new properties due to their small size (1–100 nm) [78, 79].

Because protein adsorption and cell attachment on different implant surface depends both on surface chemistry and on the surface nano-scale features, the modification of implants usually involves different surface treatments. The surface is either coated with a functional film or patterned at the nano-scale [80–82]. Cell or protein adhesive groups are commonly introduced as terminal groups in self-assembled layers of functional amphiphiles. The surface patterning can be done either by coating the implant with nanoparticle films with specific dimensions or physically fabricating the implant surface with nanostructures by using template-molding methods or by using lithography to optimize the interactions between the functional coatings/nanostructured patterns and cells/tissue [21]. For example, many companies now coat bone implants with nano-scale-textured hydroxyapatite, a mineral found in bone. This hydroxyapatite coating tricks the body into incorporating the implant as though it was a real bone. Hydroxyapatite coatings can make

the implants "stickier", but to have a truly successful implant, the surrounding normal bone needs to grow around the implant [83].

Ti nanotubes are being developed by a number of groups to enhance osteointegration [84]. Silver nanoparticles have been investigated for potential use on the surfaces of orthopedic implants to fight post-operation bacterial infections due to their antimicrobial properties. One potential problem is that silver nanoparticles also inhibit the growth of osteoblasts, so fighting infection and encouraging bone growth might not be simultaneously achievable with silver [85].

Companies such as Amedica use implants composed of silicon nitride to simultaneously decrease bacterial growth and encourage the formation of bone. This could be because at the nano-scale the silicon nitride is textured in a way that attracts osteoblasts and repels bacteria.

Moreover, nanotechnology has recently generated tremendous interest for various biosensing applications. Nanotechnology can aid in functioning of biosensing materials to contact, detect, and recognize target medical signals since it can provide for materials smaller in size, better in magnetic, optical and electrical properties, and more similar to the structure of bone tissue [86]. NanoShield, a start-up company, is developing a nanosensor that can measure how well an implant is doing. Carbon nanotubes on the implant detect what kinds of cells are attached to the implant, and transmit this information through an embedded microchip. Each cell in the body has different electrical properties, and these properties can tell the nanosensor if an osteoblast, an inflammatory cell, or a bacterium is attached. A nanostructured film on the implant could then release drugs, such as antimicrobials or anti-inflammatory molecules, depending on which type of cell is detected by the nanosensor [87]. Altering the surface of implants with nanotechnology has showed great potential to improve the performance of implants and will undoubtedly make them even better in the future.

5 The Role of Surfaces in Biological Properties

5.1 The Effect of Nanotopography on Protein Adsorption

Biocompatibility is the key property of biomedical materials. The biocompatibility of a material largely depends on a series of biological responses occurring at the interface of the material's surface and a biological system. Protein adsorption on the surface of a biomaterial is the first step in these responses. The adsorbed protein layer then determines the type and extent of the subsequent responses [88]. Therefore, studies on the behavior of protein adsorption are crucial to improve the biocompatibility of materials. Protein adsorption is a complicated process influenced by various factors, including the nature of proteins themselves, the surface property of materials, and the circumstances that the materials are facing. Protein adsorption behavior could be affected by both the surface chemistry and topography

of the materials. In the past few years, the effect of surface chemical compositions on protein adsorption has been extensively studied. The influence of surface topography on protein adsorption began to receive keen interest only very recently, although it was found as early as in 1964 that a cell's behavior could be influenced by its surrounding topography. Material surfaces may have multifarious topographical structures. The characteristics of these topographic structures basically include roughness, curvature, and specific geometrical figures. By altering implant surface properties, it is possible to guide select protein adsorption, as well as control the quantity and conformation of the adsorbed proteins, allowing researchers to guide select cell adhesion on to the implant surfaces, potentially improving the success and longevity of the implant [89]. One of the most promising approaches to altering surface properties of biomaterials is decreasing the material surface feature size into the nanophase regime.

When using a nanophase material, where at least one surface feature size is less than 100 nm, implant surface properties will change (i.e., surface area, energy, topography, and charge). With the development of nanotechnology, it has been feasible to introduce nanotopography onto materials surfaces, which provides a convenient platform to investigate the behavior and mechanism of protein adsorption as well as subsequent biological responses such as cell adhesion [90–93].

Surface roughness is a common element to materials. Surface wettability and energy are significantly affected by different roughness values, which will certainly further influence the behavior of biological molecules contacting the material surface. Research outcomes concerning the effect of nanometer scale roughness on the amount of adsorbed proteins are to some extent inconsistent. Cai et al. investigated protein adsorption on different material surfaces with diverse roughnesses. Their results indicated that there were no linear relationship between roughness and the amount of adsorbed proteins and roughness had no significant effect on the amount of adsorbed proteins [94].

However, Rechendorff et al. showed that with the augment of tantalum surface roughness, though surface area also approximately increased by 20%, the corresponding saturation amount of fibrinogen markedly increased by about 70%, which evidently indicated that the amount of adsorbed protein was influenced by surface roughness [95]. We believe that the aforementioned different conclusions were associated with the substrates, the sorts of proteins, and the methods to test protein adsorption. Therefore, further study is needed to find out the effect of surface roughness on the amount of adsorbed protein. A linear regression model was initially developed by Khang et al. to relate surface topography and wettability with protein adsorption, which is now commonly used to predict the size of nano-scale features that should be incorporated on medical devices to improve their performance [96].

Surface energy expressed as a general equation by a linear function of a roughness factor and with a coupling constant was given as:

$$E_{s(reff)} = E_{0,s} + \rho \times r_{eff}$$

Here, r_{eff} is the effective roughness, ρ is the coupling constant and $E_{0,s}$ is the initial surface energy not related to nano-scale roughness (flat or very smooth surface $r_{eff} \sim 0$). Thus, individual factors (roughness or surface energy) on protein adsorption could be easily demonstrated as:

$$F_{adsorption}\left(r_{eff}, E_s\right) = \alpha \times r_{eff} + \beta \times E_s$$

Here, $F_{adsorption}$ is the protein adsorption, E_s is surface energy of the material, and α and β are coupling constants. When the two equations are coupled, it is possible to define protein adsorption as a function of only r_{eff}, as shown below:

$$F_{adsorption}\left(r_{eff}\right) = A \times r_{eff} + \beta \times E_s$$

Here, $A = \alpha + \beta \times \rho$

Coupling A indicates the contribution of nanophase surface roughness and β indicated the contribution of ground surface energy on protein adsorption on to a biomaterial surface [97]. Since protein adsorption behavior could be affected by nanometer-scale roughness, more attention should be paid to the effect of roughness on the biocompatibility of biomaterials. In addition, biomedical materials or devices have defined all geometrical figures. Therefore, the effect of such geometrical figures on protein and other biological molecules should also be investigated, so that biomedical materials or devices could be rationally designed. Galli et al. produced nanometer groove structures with dimensions similar to protein size on silicon and titanium surfaces. They chose protein A and F-actin as two different model proteins. The results suggested that on silicon surfaces, the amount of adsorbed F-actin was lower than on nanometer groove structures than on plane surfaces and F-actin was inclined to adsorb along with the nanometer groove structure; on titanium surfaces, the adsorption density of F-actin was related to the height of surface topography. Different from F-actin, there was no difference in protein adsorption behavior and activity on different surface topographies for protein A [98].

The above results suggested that different proteins with dissimilar characteristics (i.e., shape and size) have distinct responses to diverse nanotopographies. Sutherland et al. prepared pits with diameters of 40 nm and a depth of 10 nm on material surfaces. Quartz crystal microbalance experiments indicated that the amounts of adsorbed fibrinogen on plane and nanopits surfaces were similar. In order to test whether nanopit structures affected the biological activity of adsorbed fibrinogen, both plane and nanopits surfaces with preadsorbed fibrinogen were incubated in unactivated platelets solutions, and it was found that more platelets adhered on the nanopit surface. They presumed that this was a result of different conformations and orientations adopted by fibrinogen on different surface topographies. The conformation and orientation on nanopits surface were favorable for the combination of ligands in fibrinogen and receptors on platelet membranes, leading to more adhered platelets on nanopit surfaces [99]. In summary, protein adsorption behavior could be influenced by surface nanotopography. However, protein adsorption is merely the

first step in a series of biological responses after a biomaterial comes in contact with a biological environment, and the subsequent responses, such as cell attachment and platelet adhesion, will determine the ultimate biocompatibility of biomaterials. Thus, research about the effect of surface nanotopography on protein adsorption is just beginning. The influence of absorbed protein conformation, orientation, and structure induced by surface nanotopography on subsequent cell behavior deserves further investigation. In addition, surface topography and surface chemical compositions are two sides of one coin. Each side is relatively independent, affecting the adsorption behavior of proteins and other biological molecules, but also supplemental to each other, determining the biocompatibility of all the biomaterials. Therefore, chemical modification on an optimized surface topography, or constructing a topography structure on the surface with a specific chemical composition is another trend, so that most desirable material surface properties can be obtained by a synergic effect of surface topography and chemical composition.

5.2 The Effect of Nanotopography on Cellular Functions

The rapid development of fabrication and processing technologies in the past two decades has enabled researchers to introduce nano-scale features into materials, which, interestingly, have been shown to greatly regulate the behavior and fate of biological cells. In particular, important cell responses (such as adhesion, proliferation, differentiation, migration, and filopodial growth) have all been correlated with material nanotopography [100]. Given its great potential, intensive efforts have been made, both experimentally and theoretically, to understand why and how cells respond to nano-scale surface features. It is important to emphasize that many natural tissues are essentially composed of nano-scale structures. For example, bone possesses a complex organic-inorganic nanocomposite structure. The organic phase is mainly composed of type I collagen, which is arranged into nanofibers ranging from 50 to 500 nm in diameter. The inorganic phase consists of non-stoichiometric hydroxyapatite crystals with lengths of about 100 nm, widths of 20–30 nm and thicknesses of 3–6 nm, which are embedded between the collagen fibers. Therefore, by mimicking their natural nanostructure on implants, biomaterials might be able to enhance/regulate the functions of specific cells or tissues.

This principle has been demonstrated through the wide application of synthetic polymers (poly (lactic-co-glycolic) acid (PLGA), PDMS, etc.) and metals (Ti, stainless steel, etc.) in clinical practice as well as in laboratory research. In particular, the capabilities of surfaces with ordered nanofeatures in regulating the behavior, including adhesion, growth, alignment and elongation, of cells have been convincingly demonstrated. For instance, it was found that PLGA surfaces with 200-nm spherical topographies promote the adhesion of endothelial and smooth muscle cells, compared to smooth PLGA substrates. On the other hand, as reported by Smith et al., the presence of nanometer scale roughness on PLGA surfaces, prepared by sodium hydroxide-etching, inhibits the adhesion of fibroblasts (cells that form connective

tissue) while, interestingly, promotes osteoblast adhesion [101]. These properties may be definitely due to the altered surface energy one gets with different nano-scale features which in turn alters initial protein adsorption.

Anodization is one of the simplest surface modification processes used to create nanotextured and nanotubular features on metal oxides, which has been shown to improve bone formation. Anodization of Ti leads to the formation of titanium dioxide (TiO_2) nanotubes on the surface, and the presence of these nanotubes mimics the natural nano-scale features of bone. It was found that increasing the anodization voltages from 5 V, 10 V, 15 V to 20 V resulted in a Ti surface that contained nanotubular-like structures with an inner diameter from 20 nm, 40 nm, 60 nm to 80 nm, respectively.

Numerous studies have shown improved osteoblast attachment, migration, and proliferation on nanotubular anodized Ti surfaces. Most importantly, it was also demonstrated that decreased numbers of macrophages adhered to nanotubular Ti surfaces compared with unanodized conventional nanosmooth Ti (controls), which should be strongly considered to improve orthopedic implant efficacy since it implies reduced inflammation [102]. 316L stainless steel with tunable nanometer pit sizes (0, 25, 50, and 60 nm) were fabricated by Ni et al. with an anodization procedure. They found that compared with unanodized (that is, nano-smooth) surfaces, the 50 and 60 nm diameter nano-pit surfaces dramatically enhanced initial human dermal fibroblast attachment and growth for up to 3 days in culture. Such nanopit surfaces can be designed to support fibroblast growth and, thus, improve the use of 316L stainless steel for various implant applications (such as for enhanced skin healing for amputee devices and for percutaneous implants) [103].

It is not difficult to infer that different types of cells will probably respond distinctly to the same surface nanotopography. This kind of cell type-specific response suggests that nanotopography might be able to selectively mediate the functions and activities of various cells. This selective mediation on different cell types renders the possibility of suppressing the activities of undesired cells while simultaneously promoting the response of target cells.

5.3 The Effect of Nanotopography on Bacterial Attachment

Billions of dollars are spent annually worldwide to combat the adverse effects of bacterial attachment and biofilm formation on biomaterials for medical applications such as catheters, artificial heart valve replacements, and orthopedic and dental implants. While advances in the fabrication of antifouling surfaces have been reported recently, a number of the essential aspects responsible for the formation of biofilms remain unresolved, including the important initial stages of bacterial attachment to a substrate surface. The reduction of bacterial attachment to surfaces is a key concept in the prevention or minimization of biofilm formation. The chemical and physical characteristics of both the substrate and bacteria are important in understanding the attachment process, but substrate modification is likely the most

practical route to enable the extent of bacterial attachment taking place to be effectively controlled. There is increasing evidence that bacterial attachment and subsequent biofilm formation are significantly impacted by surface topography. Understanding the behavior of bacteria on nanostructured materials is a key factor for designing surfaces that are capable of controlling bacterial colonization. A number of studies have indicated that bacteria are able to sense topographical nanofeatures, however, the exact mechanisms that then regulate the bacterial response to the nanotopography have not been reported [104–107].

For surfaces with topographical features at the micrometric scale, comparable with the size of prokaryotic cells, cells tend to position themselves such that they maximize contact area with the surface, which favors attachment. Surfaces with topographical features of dimensions much smaller than microbial cells, in the submicrometric or nanometric range, have been reported to inhibit attachment by reducing the contact area between bacteria cells and the surface. In addition, surface topography at the nano-scale can create energetic situations unfavorable for bacterial attachment, and induce repulsive surface-bacteria interaction forces that impair attachment and subsequent biofilm formation [108]. Nature provides some clues to preventing microbial colonization by constructing surfaces with nanostructures. For example, cicada wing surfaces (nanopillared surfaces; each nanopillar is approximately 200 nm in height, 70 nm in diameter, and the pillars are 170 nm apart from center to center) (Fig. 3) have been demonstrated to be bactericidal to Gram-negative bacteria (i.e., *Pseudomonas aeruginosa*), which were exclusively due to the surface nanostructure of the wing rather than a surface chemical effect. It has been suggested that the attachment of bacterial cell membranes onto the cicada wing surface lead to a stretching effect on the membrane, consequently leading to cell membrane rupture and death. It has also been shown that Gram-positive bacteria, whose bacterial cell membrane is generally much thicker than that of Gram-negative bacteria, are not killed by this mechanism (Gram-negative bacteria contain a layer of peptidoglycan which is 2–3 nm thick, whereas Gram-positive bacteria possess a thicker layer of 20–80 nm) [109].

Puckett et al. explored the adhesion of multiple bacteria species well known to lead to orthopedic implant infection on nanotubular, nanotextured, nanorough, and

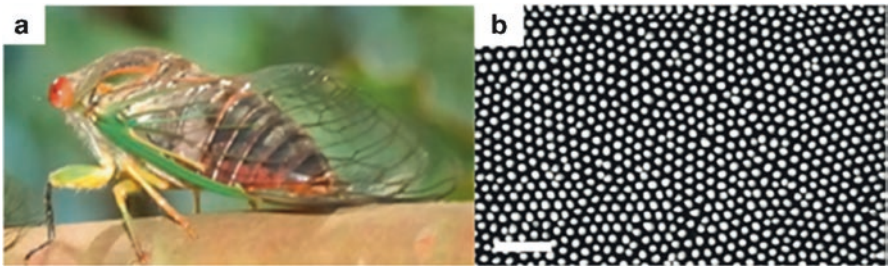

Fig. 3 Cicada wing surface: a natural antibacterial surface that arises as a result of the surface nano-topography. (**a**) Photography of the cicada and (**b**) scanning micrograph of the hexagonal arrangement of the nanopillars on the cicada wing surfaces, scale bar = 200 nm

conventional Ti. Results demonstrated the decreased adhesion of *S. aureus*, *S. epidermidis*, and *P. aeruginosa* (bacteria that limit orthopedic implant function and efficacy) on nanorough Ti surfaces created through electron beam evaporation while nanotubular and nanorough Ti created through anodization resulted in an increase of bacteria attachment. This research indicated that it was possible to decrease bacteria adhesion and growth through careful selection of nanometer surface properties [32, 110]. Interestingly, in a recent investigation by Epstein et al., they found that bacterial adhesion and biofilm growth varied depending on the geometry of nanostructure. Their work could lead to a more nuanced understanding of what makes a surface less inviting to bacteria [111].

All of these studies confirm that the bacteria sense the nanotopography of the surface and adhere less on specific nanostructures.

6 Nanofabrication Techniques

In the emerging and popular field of nanostructured materials, structural manipulations at an atomic, molecular and/or supramolecular length scales are an essential pathway permitting the design of novel materials. Nanofabrication techniques have revolutionized the pharmaceutical and medical fields as they offer the possibility for highly reproducible mass-fabrication of systems with complex geometries and functionalities. There are several approaches to achieve the desired structures to explore unique properties that emerge at the nanometer scale [112]. In general, methods used to generate nano-scale structures and nanostructured materials can be characterized as "top-down" and "bottom-up". In simple terms, the "top-down" approach uses various methods such as lithography to pattern nano-scale structures, while the "bottom-up" approach builds a material from units, usually via self-organization or self-assembly of atoms and molecules, at much smaller scales. Several important and frequently used fabrication techniques for generating nanotopography are introduced in this part of the chapter [113].

6.1 Nanolithography

Nanolithography is considered to be the most advanced technique in patterning ultra-high-resolution patterns of arbitrary shapes to a minimum feature size of just a few nanometers. Nanolithography uses lights, charged ions, or electron beams to transfer the geometric pattern from a pre-made photomask to a photosensitive layer coated on the target material, and then relies on a series of post-treatments to chemically engrave the transferred pattern into the material or allow the deposition of new compounds along the pattern. Nanolithography techniques may include photolithography, electron-beam lithography, nanoimprint lithography, scanning probe lithography, X-ray lithography, etc. [114]. Photolithography is basically a

conventional and classical technique and it is also termed as optical lithography or UV lithography. Photolithography basically utilizes the exposure of photo-resist to ultraviolet (UV) light to obtain the desired pattern. Generally, the commonly used photo-resist is PMMA. The photo mask usually consists of the opaque features on a transparent substrate (e.g., quartz, glass) to make an exposure on a photo-resist. The exposed area of the photo-resist that breaks down, which results in increased solubility in a chemical solution, is called a developer. Subsequently, the exposed photo-resist is removed to form the desired photo-resist pattern. The process of photolithography is less expensive and highly efficient in fabricating extremely small incisions on a substrate. A single beam of UV light is sufficient for etching patterns. However, there are also some disadvantages, such as processing under a clean room environment (free from all liquids, contaminants and environmental hazards) and requiring a completely flat substrate [115].

Electron beam lithography or so-called E-beam lithography utilizes an accelerated beam of electrons to scan on the surface of a resist (PMMA) with the diameter as small as a couple of nanometers in a layer-by-layer fashion to form a desired pattern. E-beam lithography provides better resolution and greater accuracy than photolithography. It has a demonstrated 10 nm lithography resolution. However, E- beam lithography also has certain disadvantages including expensive machines, a complicated system, and time-consuming processes [116]. X-ray lithography is an extension to photolithography. The only difference is that X-ray lithography utilizes X-rays to irradiate the resist instead of the UV light in the case of photolithography. By employing electromagnetic radiation of wavelengths in the range of 0.1–10 nm, X-ray lithography can be extended to a resolution of 15 nm [35]. The diffraction limit of photolithography is overcome by X-ray lithography because of its shorter wavelength and ability to produce small feature size objects. However, the X-ray lithography tools are rather expensive and their ability to mass-produce sub-50 nm structures has yet to be demonstrated [117]. The nanolithography techniques are summarized in Tables 1, 2 and 3.

Studies have shown increased bone growth as well as reduced infection when titanium screws were coated with titania using e-beam and inserted into a rat amputee model (Fig. 4).

A number of different procedures including molding and embossing have been developed for patterning nano-scale structures. Molding involves curing a precursor (usually a monomer or a prepolymer) against a topographically patterned substrate.

Table 3 Summary of nanolithography techniques

Nanolithography techniques	Feature size	Throughput	Advantages	Disadvantages
Photo-lithography	~Micro	Very high	Less expensive; Highly efficient	Clean room processing needed; Flat substrate
E-beam lithography	5 nm	Very low	Better resolution; Greater accuracy	Slow and expensive; High maintenance costs; Complicated system
X-ray lithography	15 nm	Low	Print complex patterns	

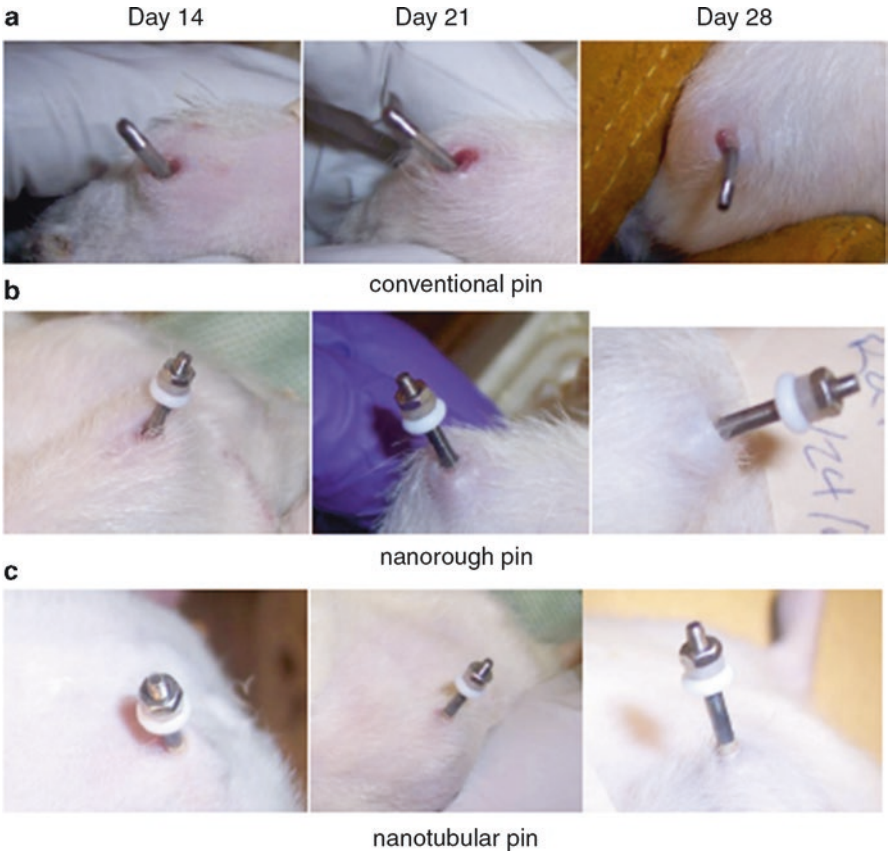

Fig. 4 Images showing wound closure when using e-beam coated titanium screws (labeled nanorough pins) and anodized nanotubular titanium screws (labeled nanotubular pins) compared to controls (labeled conventional pins). Screws were insert into resected femurs of rats for various time periods

This method of pattern transfer is used by techniques such as replica molding with a soft mask. Embossing (or imprinting) techniques transfer a mold with a structured topography into an initially flat polymer film [112, 113]. Nanoimprint lithography refers to the pressure-induced transfer of a topographical pattern from a rigid mold (typically silicon) into a thermoplastic polymer film heated above its glass-transition temperature. Another term for this method is "hot embossing" since the process involves heating the molded polymer above its glass-transition temperature. For example, to transfer the pattern from a mold into a thin polymer film of PMMA by nanoimprinting lithography requires heating the polymer film above 110 °C.

Nanoimprint lithography can mold a variety of polymeric materials and pattern features as small as 5 nm and aspect ratios up to 20 (height-to-width). One of the important issues still to be resolved is the useful lifetime of the mold. Presently,

nanoimprint molds require replacement after 50 consecutive imprints. Heating and cooling cycles, and high pressures applied during embossing, produce stress and wear on the nanoimprint molds. The high viscosity of the polymer films is another challenge for nanofabrication using this technique [118]. Techniques that prepare a soft mold or stamp by casting a liquid polymer precursor against a topographically patterned master are commonly referred to as soft lithography. A number of polymers could be used for molding [119]. Elastomers are a versatile class of polymers for replication of a topographical master. The most widely implemented and successful elastomer for nanofabrication is PDMS. PDMS has a number of useful properties for nanofabrication.

This material is durable, unreactive toward most materials being patterned or molded, chemically resistant to many solvents, and transparent above a wavelength of 280 nm. Commercially available kits or precursors for this polymer can be obtained inexpensively. One of the major advantages of PDMS is that the fabrication of molds or stamps (by replica molding) is so inexpensive that sometimes the mold or stamp becomes a disposable reagent. Replica molding consists of three steps: (1) creating a topographically patterned master; (2) transferring the pattern of this master into PDMS by replica molding; and (3) fabricating a replica of the original master by solidifying a liquid precursor against the PDMS mold. PDMS can be deformed reversibly and repeatedly without permanent distortion or relaxation of the surface topography. The cured elastomer has a low surface free energy (21.6 dynes/cm^2), which allows PDMS to be easily released after molding. An important limitation of PDMS is that the tensile modulus is relatively low (1.8 MPa) and limits the replication of nano-scale features [120].

6.2 Nanofabrication by Deposition Techniques

A number of methods have been employed to prepare nano-scale thin films, including physical vapor deposition (PVD), chemical vapor deposition (CVD), and atomic layer deposition (ALD) [112, 113]. PVD is a coating method that transports physically vaporized materials from a source onto a substrate in a vacuum chamber where condensation of vapors will form a thin film with atomic- to nano-scale roughness on the surface. In comparison, CVD, as indicated by its name, is a deposition method via chemical reaction of vapors or gas phases. The CVD technique is very versatile for creating nanomaterials with multiple dimensions (from 0D to 3D), highly ordered topographies (from dots, wires to scaffolds), and complex compositions [121]. ALD is based on sequential self-terminating surface reactions of gaseous precursors producing extremely thin, high-quality, conformal films with thickness control on the atomic level at low temperatures [122]. Main features of these three deposition techniques are summarized in Table 4 [38].

Critically, ALD has been used to coat titanium medical devices with titania nanoparticles showing significantly reduce bacteria attachment and growth without using antibiotics (Fig. 5).

Table 4 Main features of three different deposition techniques: PVD, CVD and ALD

PVD	CVD	ALD
Low cost	High cost	High cost
Low temperature	High temperature	High temperature
Non uniformity	Good uniformity	Excellent uniformity
High deposition rate	Average deposition rate	Low deposition rate
Target must be tuned	Good composition control	Excellent composition control
Poor industry applicability	Excellent industry applicability	Good industry applicability

6.3 Nanofabrication by Self-assembly

The self-assembly technique belongs to a bottom-up approach to nanostructures or nanostructured materials, which relies on cooperative interactions of small components that assemble spontaneously in a predefined way to produce a larger structure in two or three dimensions. There are mainly two types of self-assembly: (1) unguided self-assembly, where individual components interact to produce a larger structure without the assistance of external forces or spatial constraints, and (2) template-assisted self-assembly, where individual components interact with each other and an external force or are spatially constrained to create a desirable orientation of nanostructures [112, 113]. One of the most appealing aspects of self-assembly is the spontaneous assembly of components into a desired structure. Examples of materials fabricated using unguided self-assembly include self-assembled monolayers and structures that self-assemble from block copolymers and nanoparticles. However, this approach is not widely used for nanofabrication since it is presently unable to produce structures with precise spatial positioning and arbitrary shapes with a low concentration of defects and functionality that can be achieved using conventional nanofabrication.

By templating self-assembly it is possible to introduce an element of pattern into the self-assembled structure and sometimes increase the order of the self-assembled structure. Self-assembly can be directed using surface topography, electric and magnetic fields, or shear forces. Template-assisted self-assembly is an alternative to unguided self-assembly for the controlled fabrication of patterned structures at the nano-scale [123]. The nanofabrication by self-assembly is inherently advantageous over conventional technologies in two aspects: one is the ability to construct structures in true nano-scale; another is to make nanostructures cheaply and simply. However, there is a long way to go before this technology can be implemented in a large scale by industry.

It is important to note that self-assembled materials have demonstrated antibacterial properties as well. Specifically, self-assembled cationic peptide amphiphiles have been shown to significantly decrease bacteria growth, both gram-positive and gram-negative at low concentrations (Fig. 6) [114].

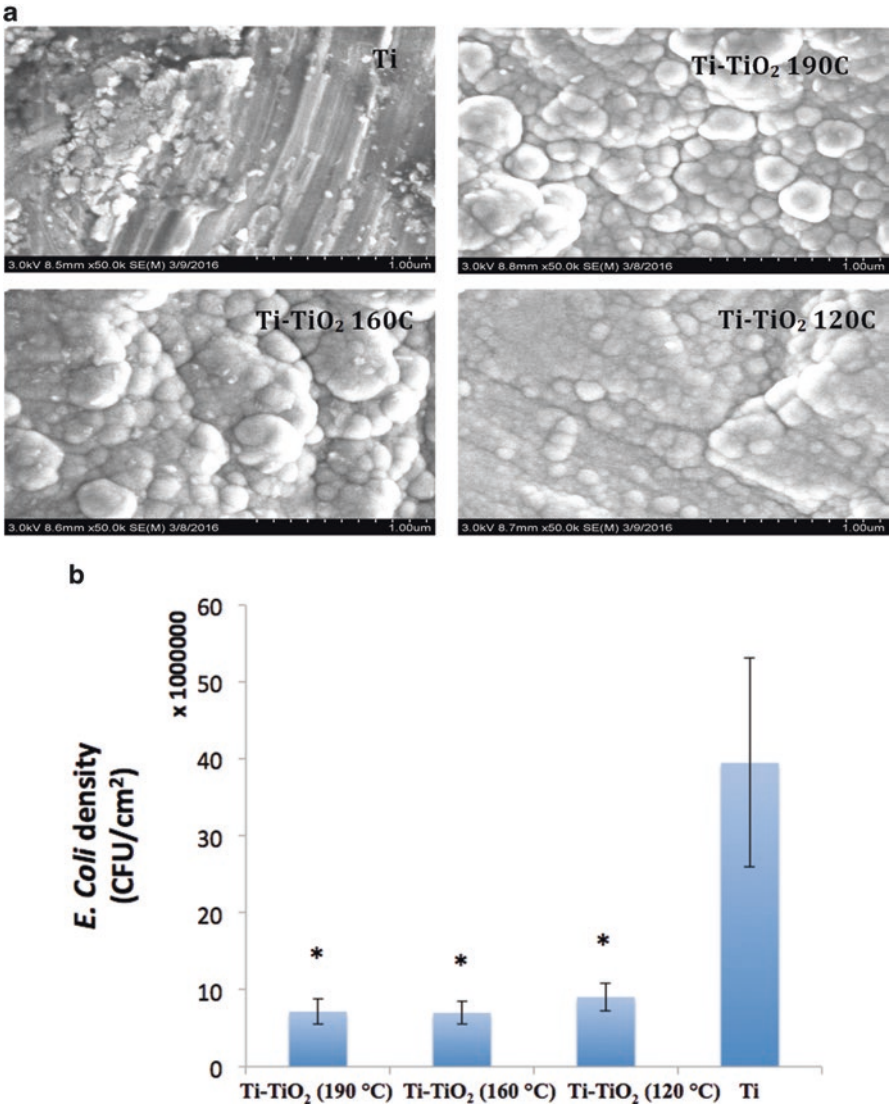

Fig. 5 (**a**) SEM images of ALD titania coated on titanium medical implants and (**b**) E. coli colony forming units (CFU) on such samples after 24 h. Data = mean ± SEM; N = 3; *p < 0.01 compared to Ti control (untreated). Temperatures refer to ALD heating conditions

7 Sensors

Of course, with all of the nanomaterials that have been developed to reduce bacteria function, a critical question still remains: can we develop the next generation of biomaterials to sense bacteria presence before an infection occurs. In this manner, researchers have created a sensor grown out of titanium based hip implants [124].

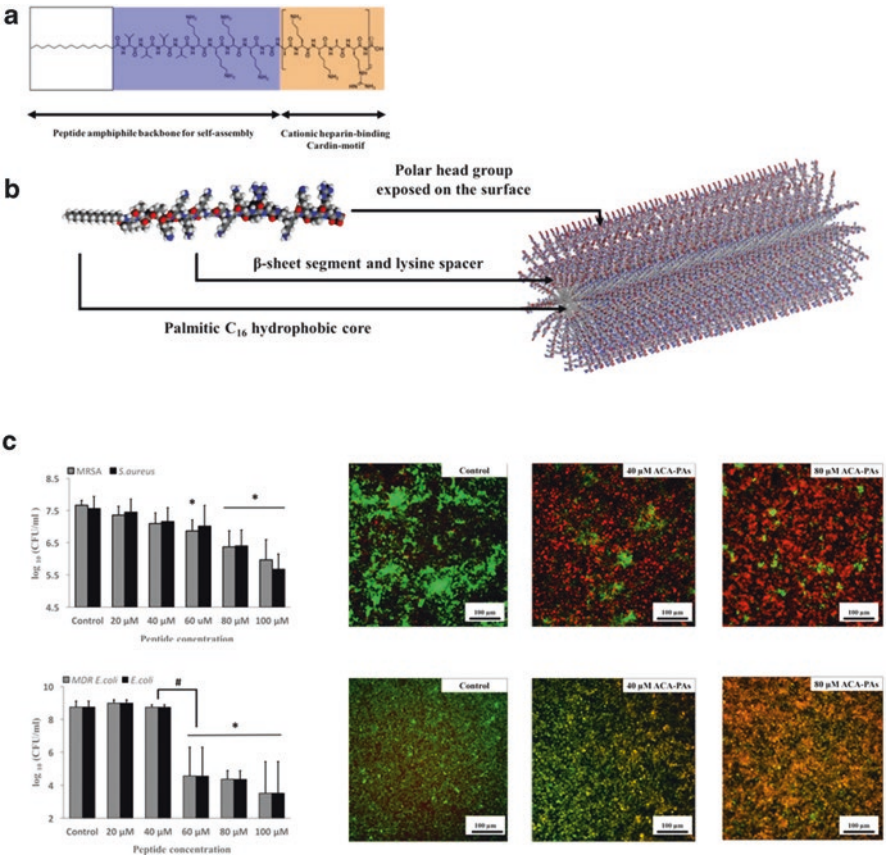

Fig. 6 Self-assembled cationic peptide amphiphiles that decrease bacteria growth. (**a**) The structure of a cationic peptide amphiphile, (**b**) a molecular simulation of the cationic peptide amphiphile, and (**c**) colony forming assay (CFU) results after culturing MRSA and multi-drug resistance bacteria (MDR) *E. coli* (left) and fluorescence microscopy results (right) in the presence of the self-assembled cationic peptide amphiphiles for 24 h. Red = dead bacteria and green = live bacteria. #, * $p < 0.01$

This titanium based implant was first anodized to possess nanotubes from which carbon nanotubes were grown using chemical vapor deposition. The carbon nanotubes can then measure the resistance of the cells that attach to the implant, send via radio frequency such information to a hand-held device, and even be remotely activated to release bone growth factors, anti-inflammatory agents, or antibiotics to the site of need to ensure implant success (Fig. 7). Such electrochemical detection of biological events may be the future of nanomaterial use in medical devices as they can sense in real time biological events depending on that individual's response to the implant to promote success, perhaps the true definition of personalized medicine.

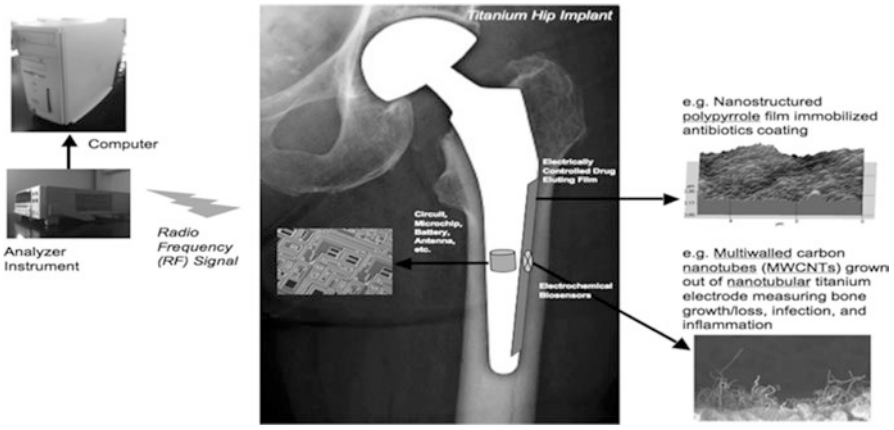

Fig. 7 Electrochemical detection of bacteria, inflammation, or bone growth surrounding a hip implant. The intelligent hip implant utilizes carbon nanotubes grown out of anodized tubular titanium to detect what type of cell attached and tissue is forming. Further, it sends such information to a hand-held device and can be remotely activated to release bone growth factors, anti-inflammatory agents, and/or antibiotics to ensure implant success

8 Conclusions

This chapter provides promise for the future of using nanotechnology to reduce orthopedic implant infections. Impressively, such results have been obtained without using antibiotics, and, thus, such approaches do not contribute to the growing concern of antibiotic resistance. Moreover, such approaches utilize mathematical equations to predict the size of nano-scale features that should be placed on medical devices to alter surface energy to in turn reduce bacteria growth. By changing surface roughness at the nano-scale and not changing surface chemistry, quicker FDA approval should ensue. Lastly, this chapter ends with a forward-thinking approach to design orthopedic implants that can both sense bacteria and be activated to kill them on-demand. In all approaches, it is clear that nanotechnology has a bright field in orthopedics.

References

1. Khan W, Muntimadugu E, Jaffe M, Domb AJ. Implantable medical devices: focal controlled drug delivery. In: Domb A, Khan W, editors. Advances in delivery science and technology. Boston, MA: Springer; 2014.
2. Regar E, Sianos G, Serruys PW. Stent development and local drug delivery. Br Med Bull. 2001;59:227–48.
3. Orthopedic implants market analysis, by application (spinal fusion, long bone, foot & ankle, craniomaxillofacial, joint replacement, dental), and segment forecasts to 2024. Grand View Research; 2016.

4. Wischke C, Lendlein A. Designing multifunctional polymers for cardiovascular implants. Clin Hemorheol Microcirc. 2011;49:347–55.
5. Cardiovascular implants market analysis & trends-device, procedure, disease condition-forecast to 2025. Research and Markets; 2016.
6. Catheters market analysis by product (cardiovascular, urology, intravenous, neurovascular and specialty catheters) and segment forecasts to 2020. Grand View Research; 2014.
7. Biomaterials market for implantable devices (material type-metals, polymers, ceramics and natural; applications-cardiology, orthopedics, dental, ophthalmology and others)-global industry analysis, size, share, growth, trends and forecast 2013-2019. Transparency Market Research; 2014.
8. Grainger DW. All charged up about implanted biomaterials. Nat Biotechnol. 2013;31:507–9.
9. Saini M, Singh Y, Arora P, Arora V, Jain K. Implant biomaterials: a comprehensive review. World J Clin Cases. 2015;3:52–7.
10. Chen Q, Thouas GA. Metallic implant biomaterials. Mater Sci Eng R-Rep. 2015;87:1–57.
11. Teo AJT, Mishra A, Park I, Kim Y-J, Park W-T, Yoon Y-J. Polymeric biomaterials for medical implants and devices. ACS Biomater Sci Eng. 2016;2:454–72.
12. Matassi F, Nistri L, Paez DC, Innocenti M. New biomaterials for bone regeneration. Clin Cases Miner Bone Metab. 2011;8:21–4.
13. Nablo BJ, Prichard HL, Butler RD, Klitzman B, Schoenfisch MH. Inhibition of implant-associated infections via nitric oxide release. Biomaterials. 2005;26:6984–90.
14. Mel A, Cousins BG, Seifalian AM. Surface modification of biomaterials: a quest for blood compatibility. Int J Biomater. 2012;2012:707863.
15. Gotman I. Characteristics of metals used in implants. J Endourol. 1997;11:383–9.
16. Godbole N, Yadav S, Ramachandran M, Belemkar S. A review on surface treatment of stainless steel orthopedic implants. Int J Pharm Sci Rev Res. 2016;36:190–4.
17. Holzwarth U, Thomas P, Kachler W, Göske J, Schuh A. Metallurgical differentiation of cobalt-chromium alloys for implants. Orthopade. 2005;34:1046–7. 1049–51
18. Oldani C, Dominguez A. Titanium as a biomaterial for implants. In: Fokter S, editor. Recent advances in arthroplasty. London: InTech; 2012.
19. Rack HJ, Qazi JI. Titanium alloys for biomedical applications. Mater Sci Eng C. 2006;26:1269–77.
20. Hofmann J, Michel R, Holm R, Zilkens J. Corrosion behaviour of stainless steel implants in biological media. Surf Interface Anal. 1981;3:110–7.
21. Flecka C, Eiflerb D. Corrosion, fatigue and corrosion fatigue behaviour of metal implant materials, especially titanium alloys. Int J Fatigue. 2010;32:929–35.
22. Nuss KMR, Rechenberg B. Biocompatibility issues with modern implants in bone-a review for clinical orthopedics. Open Orthop J. 2008;2:66–78.
23. Plecko M, Sievert C, Andermatt D, Frigg R, Kronen P, Klein K, Stübinger S, Nuss K, Bürki A, Ferguson S, Stoeckle U, Rechenberg B. Osseointegration and biocompatibility of different metal implants - a comparative experimental investigation in sheep. BMC Musculoskelet Disord. 2012;13:32.
24. Rickert D. Polymeric implant materials for the reconstruction of tracheal and pharyngeal mucosal defects in head and neck surgery. GMS Curr Top Otorhinolaryngol Head Neck Surg. 2009;8:Doc06.
25. Maitz MF. Applications of synthetic polymers in clinical medicine. Biosurf Biotribol. 2015;1:161–76.
26. Kurtz SM. UHMWPE biomaterials handbook: ultra high molecular weight polyethylene in total joint replacement and medical devices. Philadelphia: Elsevier Science; 2009.
27. Li X, Kruger JA, Jor JWY, Wong V, Dietz HP, Nash MP, Nielsen PMF. Characterizing the ex vivo mechanical properties of synthetic polypropylene surgical mesh. J Mech Behavior Biomed Mater. 2014;37:48–55.

28. Bergmann PA, Becker B, Mauss KL, Liodaki ME, Knobloch J, Mailänder P, Siemers F. Titanium-coated polypropylene mesh (TiLoop Bra) an effective prevention for capsular contracture? Eur J Plastic Surg. 2014;37:339–46.
29. Terrada C, Julian K, Cassoux N, Prieur AM, Debre M, Quartier P, LeHoang P, Bodaghi B. Cataract surgery with primary intraocular lens implantation in children with uveitis: long-term outcomes. J Cataract Refract Surg. 2011;37:1977–83.
30. Rivkin AA. Prospective study of non-surgical primary rhinoplasty using a polymethylmethacrylate injectable implant. Dermatol Surg. 2014;40:305–13.
31. Leigh JA. Use of PMMA in expansion dental implants. J Biomed Mater Res. 1975;9:233–42.
32. Eyerer P, Jin R. Influence of mixing technique on some properties of PMMA bone cement. J Biomed Mater Res A. 1985;20:1057–94.
33. Panahi-Bazaz M-R, Zamani M, Abazar B. Hydrophilic acrylic versus PMMA intraocular lens implantation in pediatric cataract surgery. J Ophthalmic Vis Res. 2009;4:201–7.
34. Kurtz SM, Devine JN. PEEK biomaterials in trauma, orthopedic, and spinal implants. Biomaterials. 2007;28:4845–69.
35. Garcia-Gonzaleza D, Jayamohanb J, Sotiropoulosc SN, Yoond S-H, Cookd J, Siviourd CR, Ariasa A, Jérusalem A. On the mechanical behaviour of PEEK and HA cranial implants under impact loading. J Mech Behav Biomed Mater. 2017;69:342–54.
36. Aronoff MS. Market study: biomaterials supply for permanent medical implants. J Biomater Appl. 1995;9:205–61.
37. Chugay N, Chugay P, Shiffman M. Body implants: overview. In body sculpting with silicone implants. New York: Springer; 2014. p. 1–12.
38. Rahimi A, Mashak A. Review on rubbers in medicine: natural, silicone and polyurethane rubbers. Plast Rubber Compos. 2013;42:223–30.
39. Qin Y, Howlader MM, Deen MJ, Haddara YM, Selvaganapathy PR. Polymer integration for packaging of implantable sensors. Sensors Actuators B Chem. 2014;202:758.
40. Geeroms B, Laleman W, Laenen A, Heye S, Verslype C, van der Merwe S, Nevens F, Maleux G. Expanded polytetrafluoroethylene-covered stent-grafts for transjugular intrahepatic portosystemic shunts in cirrhotic patients: Long-term patency and clinical outcome results. Eur Radiol. 2017;27(5):1795–803.
41. Masood R, Hussain T, Umar M, Azeemullah, Areeb T, Riaz S. In situ development and application of natural coatings on non-absorbable sutures to reduce incision site infections. J Wound Care. 2017;26:115–20.
42. Roohpour N, Wasikiewicz JM, Moshaverinia A, Paul D, Grahn MF, Rehman IU, Vadgama P. Polyurethane membranes modified with isopropyl myristate as a potential candidate for encapsulating electronic implants: a study of biocompatibility and water permeability. Polymers. 2010;2:102–19.
43. de la Peña-Salcedo J, Soto-Miranda M, Lopez-Salguero J. Back to the future: a 15-year experience with polyurethane foam-covered breast implants using the partial-subfascial technique. Aesthetic Plast Surg. 2012;36:331–8.
44. Adeosun SO, Lawal GI, Gbenebor OP. Characteristics of biodegradable implants. J Miner Mater Charact Eng. 2014;2:88–106.
45. Gogolewski S. Bioresorbable polymers in trauma and bone surgery. Injury. 2000;31:D28–32.
46. Santos AR Jr. Bioresorbable polymers for tissue engineering. In: Eberli D, editor. Tissue engineering. London: InTech; 2010.
47. Andreiotelli M, Wenz HJ, Kohal RJ. Are ceramic implants a viable alternative to titanium implants? A systematic literature review. Clin Oral Implants Res. 2009;20(Suppl 4):32–47.
48. Al-Sanabani JS, Madfa AA, Al-Sanabani FA. Application of calcium phosphate materials in dentistry. Int J Biomater. 2013;2013:876132.
49. Özkurt Z, Kazazoğlu E. Zirconia dental implants: a literature review. J Oral Implantol. 2011;37:367–76.
50. Maccauro G, Bianchino G, Sangiorgi S, Magnani G, Marotta D, Manicone PF, Raffaelli L, Rossi Iommetti P, Stewart A, Cittadini A, Sgambato A. Development of a new

zirconia-toughened alumina: promising mechanical properties and absence of in vitro carcinogenicity. Int J Immunopathol Pharmacol. 2009;22:773–9.
51. Anderson JM, Rodriguez A, Chang DT. Foreign body reaction to biomaterials. Semin Immunol. 2008;20:86–100.
52. Eiff CV, Jansen B, Kohnen W, Becker K. Infection associated with medical devices. pathogenesis, management, prophylaxis. Drugs. 2005;65:179–214.
53. Klevens RM, Edwards JR, Richards CL Jr, et al. Estimating health care-associated infections and deaths in U.S. hospitals, 2002. Public Health Rep. 2007;122:160–6.
54. Morais JM, Papadimitrakopoulos F, Burgess DJ. Biomaterials/tissue interactions: possible solutions to overcome foreign body response. AAPS J. 2010;12:188–96.
55. Onuki Y, Bhardwaj U, Papadimitrakopoulos F, Burgess DJ. A review of the biocompatibility of implantable devices: current challenges to overcome foreign body response. J Diabetes Sci Technol. 2008;2:1003–15.
56. Dekel N, Gnainsky Y, Granot I, Racicot K, Mor G. The role of inflammation for a successful implantation. Am J Reprod Immunol. 2014;72:141–7.
57. Tang L, Eaton JW. Inflammatory responses to biomaterials. Am J Clin Pathol. 1995;103:466–71.
58. Kolaczkowska E, Kubes P. Neutrophil recruitment and function in health and inflammation. Nat Rev Immunol. 2013;13:159–75.
59. Ueha S, Shand FHW, Matsushima K. Cellular and molecular mechanisms of chronic inflammation-associated organ fibrosis. Front Immunol. 2012;3:71.
60. Stroncek JD, Reichert WM. Overview of wound healing in different tissue types; Indwelling neural implants: strategies for contending with the in vivo environment. Boca Raton, FL: CRC Press/Taylor & Francis; 2008.
61. Jansen B, Peters G. Foreign body associated infection. J Antimicrob Chemother. 1993;32:69–75.
62. Wolcott RD, Ehrlich GD. Biofilms and chronic infections. JAMA. 2008;299(22):2682–4.
63. Hedrick TL, Adams JD, Sawyer RG. Implant-associated infections: an overview. J Long-Term Eff Med Implants. 2006;16:83–99.
64. Montanaro L, Speziale P, Campoccia D, Ravaioli S, Cangini I, Pietrocola G, Giannini S, Arciola CR. Scenery of Staphylococcus implant infections in orthopedics. Future Microbiol. 2011;6:1329–49.
65. Behzadi P, Behzadi E, Yazdanbod H, Aghapour R, Akbari Cheshmeh M, Salehian OD. A survey on urinary tract infections associated with the three most common uropathogenic bacteria. Maedica (Buchar). 2010;5:111–5.
66. New spin on gram stain bacteria. June 12, 2015. Available from: https://hemtecks.wordpress.com/2015/06/12/new-spin-on-gram-stain-bacteria/.
67. Stewart PS, Costerton JW. Antibiotic resistance of bacteria in biofilms. Lancet. 2001;358(9276):135–8.
68. Hidron AI, Edwards JR, Patel J. Antimicrobial-resistant pathogens associated with healthcare-associated infections: annual summary of data reported to the National Healthcare Safety Network at the Centers for Disease Control and Prevention, 2006-2007. Infect Control Hosp Epidemiol. 2008;29(11):996–1101.
69. Ribeiro M, Monteiro FJ, Ferraz MP. Infection of orthopedic implants with emphasis on bacterial adhesion process and techniques used in studying bacterial-material interactions. Biomatter. 2012;2:176–94.
70. Katsikogianni M, Missirlis YF. Concise review of mechanisms of bacterial adhesion to biomaterials and of techniques used in estimating bacteria-material interactions. Eur Cell Mater. 2004;8:37–57.
71. An YH, Friedman RJ. Concise review of mechanisms of bacterial adhesion to biomaterial surfaces. J Biomed Mater Res. 1998;43:338–48.
72. Costerton JW. Introduction to biofilm. Int J Antimicrob Agents. 1999;11(3–4):217–21.

73. Ryder MA. Catheter related infections: it's all about biofilm. Adv Pract Nurs eJournal. 2005;5:1–16.
74. Major stages of biofilm formation. Available from: http://orthobond.com/technology/nosocomial-challenge/antimicrobial-surfaces/strategy/.
75. Noimark S, Dunnill CW, Wilson M, Parkin IP. The role of surface in catheter-associated infections. Chem Soc Rev. 2009;38:3435–48.
76. Costerton JW, Stewart PS, Greenberg EP. Bacterial biofilms: a common cause of 49 persistent infections. Science. 1999;284:1318–22.
77. Williams DF. On the nature of biomaterials. Biomaterials. 2009;30:5897–909.
78. Williams D. The relationship between biomaterials and nanotechnology. Biomaterials. 2008;29:1737–8.
79. Pathan DS, Doshi SB, Muglikar SD. Nanotechnology in implants: the future is small. Univ Res J Dent. 2015;5:8–13.
80. Yang L, Liu H, Lin Y. Biomaterial nanotopography-mediated cell responses: experiment and modeling. Int J Smart Nano Mater. 2014;5:227–56.
81. Slepicka P, Kasalkova NS, Siegel J, Kolska Z, Bacakova L, Svorcik V. Nano-structured and functionalized surfaces for cytocompatibility improvement and bactericidal action. Biotechnol Adv. 2015;33:1120–9.
82. Park GE, Webster TJ. A review of nanotechnology for the development of better orthopedic implants. J Biomed Nanotechnol. 2005 Mar;1(1):18–29.
83. Ong JL, Chan DC. Hydroxyapatite and their use as coatings in dental implants: a review. Crit Rev Biomed Eng. 2000;28:667–707.
84. Lee J-K, Choi D-S, Jang I, Choi W-Y. Improved osseointegration of dental titanium implants by TiO_2 nanotube arrays with recombinant human bone morphogenetic protein-2: a pilot in vivo study. Int J Nanomedicine. 2015;10:1145–54.
85. Brennan SA, Ní Fhoghlú C, Devitt BM, O'Mahony FJ, Brabazon D, Walsh A. Silver nanoparticles and their orthopaedic applications. Bone Joint J. 2015;97-B:582–9.
86. Liu L, Webster TJ. In situ sensor advancements for osteoporosis prevention, diagnosis, and treatment. Curr Osteoporos Rep. 2016;6:386–95.
87. Sirivisoot S, Pareta RA, Webster TJ. A conductive nanostructured polymer electrodeposited on titanium as a controllable, local drug delivery platform. J Biomed Mater Res A. 2011;99:586–97.
88. Andrade JD, Hlady V. Protein adsorption and materials biocompatibility: a tutorial review and suggested hypotheses; biopolymers/non-exclusion HPLC. Advances in polymer science, vol. 79. Berlin, Heidelberg: Springer; 1986.
89. Raffaini G, Ganazzoli F. Protein adsorption on biomaterial and nanomaterial surfaces: a molecular modeling approach to study non-covalent interactions. J Appl Biomater Biomech. 2010;8:135–45.
90. Song W, Chen H. Protein adsorption on materials surfaces with nano-topography. Chin Sci Bull. 2007;52:3169.
91. Carpenter J, Khang D, Webster TJ. Nanometer polymer surface features: the influence on surface energy, protein adsorption and endothelial cell adhesion. Nanotechnology. 2008;19:505103.
92. Denis FA, Hanarp P, Sutherland DS, Gold J, Mustin C, Rouxhet PG, Dufrêne YF. Protein adsorption on model surfaces with controlled nanotopography and chemistry. Langmuir. 2002;18:819–28.
93. Scopelliti PE, Borgonovo A, Indrieri M, Giorgetti L, Bongiorno G, Carbone R, Podesta` A, Milani P. The effect of surface nanometre-scale morphology on protein adsorption. PLoS One. 2010;5:e11862.
94. Cai K, Bossert J, Jandt KD. Does the nanometre scale topography of titanium influence protein adsorption and cell proliferation? Colloids Surf B. 2006;49:136–44.
95. Rechendorff K, Hovgaard MB, Foss M, et al. Enhancement of protein adsorption induced by surface roughness. Langmuir. 2006;22:10885–8.

96. Khang D, Kim SY, Liu-Snyder P, Palmore GTR, Durbin SM, Webster TJ. Enhanced fibronectin adsorption on carbon nanotube/ poly (carbonate) urethane: Independent role of surface nano-roughness and associated surface energy. Biomaterials. 2007;28:4756–68.
97. Ercan B, Khang D, Carpenter J, Webster TJ. Using mathematical models to understand the effect of nanoscale roughness on protein adsorption for improving medical devices. Int J Nanomed. 2013;8:75–81.
98. Galli C, Coen MC, Hauert R, Katanaevc VL, Wymannd MP, Gröninga P, Schlapbacha L. Protein adsorption on topographically nanostructured titanium. Surf Sci. 2001;474:L180–4.
99. Sutherland DS, Broberg M, Nygren H, Kasemo B. Influence of nanoscale surface topography and chemistry on the functional behaviour of an adsorbed model macromolecule. Macromol Biosci. 2001;1:270–3.
100. Tsimbouri P, Gadegaard N, Burgess K, White K, Reynolds P, Herzyk P, Oreffo R, Dalby MJ. Nanotopographical effects on mesenchymal stem cell morphology and phenotype. J Cell Biochem. 2014;115:380–90.
101. Smith LL, Niziolek PJ, Haberstroh KM, Nauman EA, Webster TJ. Decreased fibroblast and increased osteoblast adhesion on nanostructured NaOH-etched PLGA scaffolds. Int J Nanomed. 2007;2:383–8.
102. Rajyalakshmi A, Ercan B, Balasubramanian K, Webster TJ. Reduced adhesion of macrophages on anodized titanium with selected nanotube surface features. Int J Nanomed. 2011;6:1765–71.
103. Ni S, Sun L, Ercan B, Liu L, Ziemer K, Webster TJ. A mechanism for the enhanced attachment and proliferation of fibroblasts on anodized 316L stainless steel with nano-pit arrays. J Biomed Mater Res B. 2014;102:1297–303.
104. Pegalajar-Jurado A, Easton CD, Crawford RJ, McArthur SL. Fabrication of a platform to isolate the influences of surface nanotopography from chemistry on bacterial attachment and growth. Biointerphases. 2015;10:011002.
105. Mitik-Dineva N, Wang J, Mocanasu RC, Stoddart PR, Crawford RJ, Ivanova EP. Impact of nano-topography on bacterial attachment. Biotechnol J. 2008;3:536–44.
106. Rizzello L, Sorce B, Sabella S, Vecchio G, Galeone A, Brunetti V, Cingolani R, Pompa PP. Impact of nanoscale topography on genomics and proteomics of adherent bacteria. ACS Nano. 2011;5:1865–76.
107. Aguayo S, Strange A, Gadegaard N, Dalbyc MJ, Bozeca L. Influence of biomaterial nanotopography on the adhesive and elastic properties of Staphylococcus aureus cells. RSC Adv. 2016;6:89347–55.
108. Feng G, Cheng Y, Wang S-Y, Borca-Tasciuc DA, Worobo RW, Moraru CI. Bacterial attachment and biofilm formation on surfaces are reduced by small-diameter nanoscale pores: how small is small enough? NPJ Biofilms Microbiomes. 2015;1:5022.
109. Pogodin S, Hasan J, Baulin VA, Webb HK, Truong VK. Biophysical model of bacterial cell interactions with nanopatterned cicada wing surfaces. Biophys J. 2013;104:835–40.
110. Puckett SD, Taylor E, Raimondo T, Webster TJ. The relationship between the nanostructure of titanium surfaces and bacterial attachment. Biomaterials. 2010;31:706–13.
111. Epstein AK, Hochbaum AI, Kim P, Aizenberg J. Control of bacterial biofilm growth on surfaces by nanostructural mechanics and geometry. Nanotechnology. 2011;22:494007.
112. Gates BD, Xu Q, Stewart M, Ryan D, Willson CG, Whitesides GM. New approaches to nanofabrication: molding, printing, and other techniques. Chem Rev. 2005;105:1171–96.
113. Biswas A, Bayer IS, Biris AS, Wang T, Dervishi E, Faupel F. Advances in top–down and bottom-up surface nanofabrication: techniques, applications & future prospects. Adv Colloid Interf Sci. 2012;170:2–27.
114. Ruchita, Srivastava R, Yadav BC. Nanolithography: processing methods for nanofabrication development. Imp J Interdiscip Res. 2016;2:275–84.
115. Ausschnitt CP, Thomas AC, Wiltshire TJ. Advanced DUV photolithography in a pilot environment. IBM J Res Dev-Opt Lithogr. 1997;41:21–37.

116. Chen Y. Nanofabrication by electron beam lithography and its applications: a review. Microelectron Eng. 2015;135:57–72.
117. Fabrizio ED, Fillipo R, Cabrini S, Kumar R, Perennes F, Altissimo M, Businaro L, Cojac D, Vaccari L, Prasciolu M, Candeloro P. X-ray lithography for micro- and nano-fabrication at ELETTRA for interdisciplinary applications. J Phys Condens Matter. 2004;16:S3517–35.
118. Zhou W, Min G, Zhang J, Liu Y, Wang J, Zhang Y, Sun Y. Nanoimprint lithography: a processing technique for nanofabrication advancement. Nano-Micro Lett. 2011;3:135–40.
119. Wolfe DB, Love JC, Whitesides GM. Nanostructure replicated by polymer molding. Dekker encyclopedia of nanoscience and nanotechnology, vol. 6. Boca Raton: CRC Press; 2004. p. 2657–66.
120. Hassanin H, Mohammadkhani A, Jiang K. Fabrication of hybrid nanostructure arrays using a PDMS/PDMS replication process. Lab Chip. 2012;12:4160–7.
121. Lukaszkowicz K. Review of nanocomposite thin films and coatings deposited by PVD and CVD technology. Nanomaterials. London: InTech; 2011.
122. Liu M, Li X, Karuturi SK, Tokb AIY, Fan HJ. Atomic layer deposition for nanofabrication and interface engineering. Nanoscale. 2012;4:1522–8.
123. Ozin GA, Hou K, Lotsch BV, Cademartiri L, Puzzo DP, Scotognella F, Ghadimi A, Thomson J. Nanofabrication by self-assembly. Mater Today. 2009;12:12–23.
124. Sirivisoot S, Webster TJ. Multiwalled carbon nanotubes enhance electrochemical properties of titanium to determine in situ bone formation. Nanotechnology. 2008;19(29):295101–13.