Biofilm-inhibiting and Osseointegration-promoting Orthopedic Implants with Novel Nanocoatings

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Abstract Orthopedic implants are medical devices surgically placed into the body to replace a missing joint or bone or to reinforce a damaged structure. However, there is up to a 28% loosening rate on cementless implanted knee joint prostheses within a 4–10-year period after implant insertion, and a 2–5% infection rate for orthopedic implants (joint prostheses and fracture fixation devices). In the USA, total hip and knee arthroplasties currently account for over one million interventions each year. Due to the enormous size of the patient population with orthopedic implants, even a currently low risk of infection or failure has not only caused many patients to suffer, but it has also incurred huge costs for the associated health care system. Therefore, there is an urgent need to develop a novel dual-functional nanocoating technology with judiciously engineered physicochemical properties to address simultaneously the two critical issues long facing orthopedic implants: lack of integration with bone tissue and biofilm-caused infections for the enhanced success of implants.

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We have generated a nanocoating showing a very promising capability of inhibiting biofilm formation by *Staphylococcus aureus* and *Staphylococcus epidermidis*, two of the most common biofilm formers on orthopedic implants, and enhancing bone conductivity simultaneously. The dual-functional nanocoatings coming out of our research demonstrated the following unique features for orthopedic implants: (1) inhibit bacterial colonization and concomitantly promote osteoblast functions; (2) generate long-lasting functionalities for practical clinical applications because these nanocoatings are dense and highly cross-linked without substances of low molecular weight; (3) provide needed abrasion resistance for orthopedic implants and ensure strong coating adhesion to the surface; and (4) improve bone integration and reduce device-related infections in the long run.

Keywords Anti-biofilm · Nanocoating · Osseointegration · Bone conductivity *Staphylococcus aureus* · *Staphylococcus epidermidis* · Low temperature plasma deposition · Dual-function · Orthopedic implants · Coating adhesion · Abrasion resistance · Surface chemistry · Contact angle · Proliferation · Differentiation Infection

Introduction

Existing Problems with Metal-Based Orthopedic Implants

Orthopedic implants, mainly made from stainless steel and titanium alloys for strength, have been increasingly used to provide fixation of bone or to replace articulating surfaces of a joint to restore the function of fractured bone segments, impaired limbs, or affected joints. However, metal, as a foreign material with a very different chemical composition from the bone (a living tissue made of minerals and collagens, more like a polymer-ceramic composites), when implanted in the human body, inevitably has very different responses at the cellular level and tissue level compared to human bone. Some of the responses are detrimental clinically and might cause significant complications and even lead to painful revision. For example, slow or incomplete osseointegration between surrounding bone and orthopedic implants could lead to implant loosening [1], and the osseointegration is affected by the differentiation of osteoblastic cells on the implant surface [2]. As another example, infection of metal implants with a biofilm has also been an unsolved problem for orthopedic implants. Bacteria in biofilms are extremely resistant to antibiotics being protected from antimicrobial agents and from host defense mechanisms [1–5]. It is even worse considering that the infection can happen during the implantation or months or years later and infection may also exist after the implants are surgically removed [6]. *Staphylococcus aureus* (*S. aureus*) and *Staphylococcus epidermidis* (*S. epidermidis*) account for approximately two thirds of infections associated with surgical implants [7]. In the USA, total hip and knee arthroplasties

currently account for almost 1.1 million interventions per year [8], and the number is expected to increase to four million annually by 2030 [9]. Around seven million Americans are living with a hip or knee replacement [10]. Approximately 10% of patients within a 10-year period require revision surgery with biofilm-related infection as the second major cause for implant failure, costing approximately $1.9 billion in the USA per year [7].

Therefore, novel strategies are urgently needed to simultaneously address the two critical issues of incomplete implant integration with bone tissue and biofilm-caused infections to further enhance the success of orthopedic implants, and to reduce associated health care costs.

Existing Strategies Modifying Implants to Prevent Biofilm Formation and Promote Osteoconductivity

Surface modification of implants has a distinguishing feature that it only modifies the top surfaces and does not alter the bulk properties. Therefore, a lot of effort has been made to modify the implant surface in order to achieve either an optimal biocompatibility, or anti-bacterial property, or both, while maintaining the excellent mechanical strength of metals. Antibiotics were successfully coated on the surface of implants to prevent biofilm formation [11–13]. However, coating a medical device with a bactericidal compound could potentially increase the risk of selecting for antibiotic-resistant pathogens in humans over time [14, 15]. Heavy metal silver was used as an anti-biofilm agent on the surfaces of biomaterials [16–18]. However, medical devices coated with silver ions or metallic silver have disappointing clinical outcomes, probably due to inactivation of metallic silver when the devices are contacting blood and the coating is wearing off [19]. Other bactericidal agents (such as furanones) have been coated on surfaces to inhibit biofilm formation [20, 21], but encountered with one critical shortcoming, that is, the surfaces could be covered by macromolecules and dead microorganisms, causing loss of their antimicrobial function [22]. The infection-resistant surface of implants was also developed by depositing a thin layer of anti-adhesion coating on implants to prevent attachment of pathogenic bacteria. Those approaches include coating of peptide-functionalized poly(L-lysine)-grafted-poly(ethylene glycol) copolymers [23], grafting of long-chain zwitterionic poly(sulfobetaine methacrylate) [24], superhydrophobic xerogel coating [25], and manufacturing of submicron-textured biomaterial surfaces [26]. However, no animal studies have been reported for those studies, and thus the in vivo anti-biofilm effectiveness remains unclear.

Rapid and complete integration between the bone and implant surface is of great importance for a successful outcome of orthopedic implantation procedures. As a result, surface modification of orthopedic implants that can improve osteoconductivity will be of great benefit to the patients receiving implants. Being the major mineral component of natural bone and structurally similar to the bone, hydroxyapatite (HA) has been used to coat orthopedic implants with the hope of

achieving a high osteogenic activity (osteoconduction and osseointegration) [27–30]. However, such coatings have not yet led to successful clinical results and displayed additional failure modes, such as delamination of coatings and undesirable osteoconductivity. Studies have shown that porous titanium orthopedic implants coated with HA could incur more severe infection in a rabbit tibiae implant model [31, 32]. Currently, none of these coatings have received FDA approval for their use on orthopedic metal implants due to unproven clinical safety and efficacy.

To bring more benefits to patients, other surface modification methods have been used to simultaneously inhibit bacterial adhesion and promote osteoblast functions [33]. Titanium surfaces modified with poly(methacrylic acid) brushes and silk sericin have shown enhanced osteoblast adhesion, proliferation, and alkaline phosphatase (ALP) activity while concomitantly reducing the adhesion of *S. aureus* and *S. epidermidis* [34]. Titanium surfaces grafted with RGD-functionalized hydrophilic polymers have also been investigated [23, 35, 36] to take advantage of the tripeptide RGD (Arg-Gly-Asp) motif present in a number of extracellular host proteins (including fibronectin and fibrinogen) that interact specifically with the integrin receptors on host cells, but is not recognized by bacteria [35]. Titanium substrates functionalized with chitosan and subsequent modification by RGD also showed substantial reduction in adherent bacteria and significantly increased osteoblast proliferation and ALP activity [37, 38]. Another approach to achieve the dual purpose of inhibiting bacterial colonization and enhancing osteoblast functions is to immobilize bone morphogenetic protein-2 (BMP-2) on titanium surfaces with either an anti-adhesive polymer or bactericidal polymer as an intermediate layer [39, 40]. Vascular endothelial growth factor (VEGF) conjugated to either bactericidal carboxymethyl chitosan (CMCS) or anti-adhesive hyaluronic acid grafted on titanium was shown to achieve similar results [41]. However, those dual-function approaches are facing the following major challenges: (1) adverse effects of friction or handling during implantation on the surface moieties of implants functionalized with proteins; (2) unfavorable development prospects from positive in vitro studies to a similar clinical outcome; and (3) uncertainties regarding the long-term performance of the implant surface modified with functional polymer coatings and growth factors that will degrade over time.

Nanocoating with Tailored Functional Groups for Biomedical Applications

Our judiciously designed dual-functional and durable nanocoatings for orthopedic implants have demonstrated promising in vitro and in vivo efficacy in inhibiting biofilm formation and concomitantly promoting osseointegration. In addition, the nanocoating is conformal (not changing the surface topography of the implant surface), durable, and tenaciously adhered to implant surfaces, suitable for orthopedic implant applications. The novel dual-functional nanocoatings of 20–30 nm in thickness are deposited on stainless steel and titanium alloy, from which orthopedic

implants are mainly made, through plasma deposition using silicon-based monomers, and its mixture with oxygen. Plasma deposition is a thin film forming process in a vacuum reactor, where thin films deposit on the surface of substrates under plasma conditions. In a plasma deposition process, monomers are introduced into a plasma reactor and get activated to produce a gaseous complex composed of highly energetic electrons, ions, free radicals and excited monomer molecules, known as the plasma state. In recent decades, plasma process has been widely used in the preparation of biomedical materials with unique performance and in the manufacturing of medical devices [42]. For instance, a new nitrogen-rich plasma-deposited biomaterial as an external coating for stent-grafts can promote healing around the implant after endovascular aneurysm repair [43]. Through plasma deposition, many appropriate functional groups, such as amine, hydroxyl, and carboxylic acid, useful for the immobilization of bioactive molecules, can be created in the deposited coatings. More importantly, these chemical groups can be put onto almost any material by choosing the right monomers and plasma processes.

Our research group has investigated plasma deposition processes extensively for various applications. One example of our work is the development of biocompatible coatings for improved thrombo-resistance and endothelialization for medical devices and implants [44–47]. It is hoped that by tailoring the chemistry and functional groups of the nanocoating, we can regulate the growth of cells and bacteria on an implant surface, thus achieving dual functions. Successful application of this nanocoating technology will help to achieve the desired outcome of rendering the implant surface more infection-resistant and more osseoconductive without the use of any antibiotics, peptides, or growth factors, as supported by the promising preliminary results of significantly less biofilm formation by *S. aureus* or *S. epidermidis* and enhanced osteoblast cell proliferation/alkaline phosphatase level on the nanocoated surfaces. Therefore, we anticipate that the novel nanocoatings could make significant impact on public health care and the area of orthopedic implants through benefiting millions of patients in the USA.

The novelty of our nanocoating technology stems from the following essential advantages: (1) it will not affect the underlying topography of the implant surface because of its nanoscale (20–30 nm) nature; (2) it is a sterile process, environmentally friendly, and cost-effective, unlike the wet chemistry processes [24–26], (3) it poses no risk for promoting antibiotic resistance because of its non-drug-based nature; and (4) it creates long-lasting functionalities due to the tenacious adhesion of the abrasion-resistant nanocoating to the implant surface through covalent chemical bonding. As demonstrated in our preliminary studies, this nanocoating approach has shown its great promise of translating positive in vitro results into in vivo efficacy and a future similar clinical outcome.

Other plasma-deposited coatings on titanium showed significantly reduced attachment of bacteria [48]. However, there was no animal study to demonstrate in vivo efficacy and no mention of osteoconductivity. The orthopedic implants with stable and durable dual functions of inhibiting biofilm and promoting osseointegration to come out of our research could become a high-impact innovation in medical implant procedures.

Experimental Setup and Methods

Fabrication of Nanocoatings with Tailored Coating Chemistry and Surface Properties

Through process optimization by means of changing plasma power level, working pressure, working gas, gas mass flow, and treatment/deposition time, the amount of surface functionalities, coating thickness and consequently the final surface properties can be adjusted and well controlled. Specifically, nanocoating optimization can be focused on balancing surface $-CH_3$ groups, which has been considered as the most important factor for reducing protein adsorption which in turn resulted in less biofilm formation as compared to a bare metal surface [49], and Si-O groups, which represent hard surfaces that could provide favorable conditions to osteoblast cells for improved proliferation even at a lower level of protein (e.g., fibrinogen) adsorption, by means of using different ratios of trimethylsilane (TMS) to O_2 in the coating process. It has been reported that a dense and rigid layer of nanoscale SiOx on the surface could promote osteogenesis of human mesenchymal stem cells (hMSCs) [50]. The nanocoating optimization strategy can be adjusted based on feedback from anti-biofilm activity and osteoconductivity studies in multiple rounds. Further work will be focused on keeping a good anti-biofilm performance already achieved with TMS nanocoatings while trying to maximize bone regrowth properties by producing denser, more rigid or harder coatings.

A bell-jar plasma reactor, which had been described in our previous work [49], was powered by a direct current (DC) power supply to generate a low temperature gas discharge plasma. TMS or its mixture with oxygen (O_2) was introduced into the plasma reactor for coating deposition. Plasma surface pretreatment using O_2 as a working gas would provide a clean and reproducible starting condition for further plasma coating deposition, by forming a well-controlled surface layer. Specifically, it was used to introduce oxygen-containing groups on the metal substrate surface for covalent chemical binding to the subsequent TMS plasma coating. The substrates included stainless steel (SS) and titanium alloy (Ti). The main operational parameters investigated included: mass flow rate of TMS (1–4 standard cubic centimeters per minute [sccm]), discharge power (5–10 W), working pressure (20–80 mTorr), and deposition time (0.2–2 min). The ratio of TMS to O_2 was varied from 1:0, 1:1, 1:2, 1:4, and 1:8 for desired coating properties by changing the surface chemistry, energy, or hardness.

It is worth mentioning that our nanocoatings are designed to be 20–30 nm thick, which could go up to 60 nm to be achieved with some combinations of process parameters for increased surface hardness, much thinner than other plasma-deposited coatings of 100–285 nm thick siloxane and fluorosiloxane on titanium [48], in which the internal stress usually inherent in thicker coatings could increase the risk of coating cracking or delamination. In our coating process, DC plasma was used, and metallic implants served as part of the cathode to ensue stronger coating adhesion and better pin-hole free coating due to positive ion bombardment to the implant

surface removing loosely bound elements, advantageous to radio frequency (RF) plasma coating processes widely used in other similar approaches for coating deposition or surface modification. Furthermore, in this DC plasma process, due to the configuration of a metallic implant as part of the cathode, every single spot of the substrate surface exposed to the plasma environment is deposited with a uniform coating of desired abrasion-resistant strength.

Characterization of Coatings and Surfaces

Surface morphology, energy, and chemical composition are often investigated to better understand how they affect surface bioactivity such as biofilm formation, osteoblast functions, and how they are correlated.

Scanning Electron Microscopy (SEM) was used to analyze the surface morphology of the nanocoated surfaces. **Atomic Force Microscopy (AFM)** was used to characterize surface roughness of coatings on SS and Ti and measure coating thickness on Si wafers. **Contact angle analysis** was used to evaluate the surface energy of nanocoatings and how surface hydrophobicity or wettability could affect bacterial and osteoblast cell attachment. **X-ray Photoelectron Spectroscopy (XPS)** was used to analyze the chemical composition of the coatings and chemical bonding states of the elements contained. The change in elemental composition of carbon, silicone, and oxygen can be correlated with surface biological activities. **Attenuated Total Reflection (ATR) Fourier Transformed Infrared (FTIR) Spectroscopy** was used to characterize functional groups on nanocoatings such as $-CH_3$, Si-O and their changes over plasma coating conditions.

Evaluation of Durability of Bioactivity and Nanocoating Integrity

The durability or stability of the bioactivity created on the substrate surface is very critical to the successful clinical application of orthopedic implants. It has to provide a long-lasting (preferably longer than 2 years) bioactivity on the surface of orthopedic implants to make a successful product. The nanocoating also has to maintain its mechanical integrity since premature delamination or insufficient abrasion resistance will lessen its benefits in clinical applications.

Bioactivity durability test in wet condition was performed in an environment simulating the conditions of medical devices when inserted or implanted in patients where the surfaces will be in contact with human body fluid. SS and Ti substrates with optimized nanocoatings were immersed in wells of 24-well cell plates filled with simulated body fluid (SBF) [51] with 10% fetal bovine serum (FBS) and then placed in an incubator (37 °C, 5% CO_2, humidified) for 1 and 2 months. The medium

(SBF + 10% FBS) was replaced every other day. At the end of each incubation period, the specimens were removed, rinsed, and utilized in the biofilm assay and osteoblast cell culture test to determine the durability of the nanocoatings.

Accelerated adhesion test was conducted using 2″×2″ SS and Ti substrates (wafers) coated with optimized nanocoatings. The coated wafers were immersed into a 60 °C water bath for 1, 3, and 10 days. Following standard ASTM D 3359, wafers at different time-points were taken out and scribed with a cross-hatch to form 100 tiny squares, and then a tape pull test was performed. The tested surfaces were inspected by both visual and optical microscopy. A rating scale of 0–100, which was the number of squares that remained after the tape test, was used. Rating of over 96 after 10 days of immersion was considered as a pass.

Abrasion resistance test: Nanocoated SS and Ti bone screws were implanted into bovine femur obtained from a local slaughter house and then removed. This procedure was repeated twice. Then those screws were rinsed with a PBS solution, dried and examined under optical microscope for any possible cracking or delamination.

Assessment of Nanocoatings with In Vitro and In Vivo Models

In vitro biofilm assay: TMS nanocoatings with desired mechanical strength, durability, and biocompatibility, as determined by the aforementioned studies, were further tested for in vitro anti-biofilm property evaluation. Bacteria were cultured on wafers that were coated with 20% (v/v) human plasma coated [52] in 24-well flat-bottomed sterile microtiter plates overnight. Bacteria (*S. aureus* NRS234 and *S. epidermidis* RP62A) in biofilms were counted by a plate counting method following being dispersed by ultrasonication, as previously reported [49].

Small animal treatment: The mouse bone implant infection model allowed us to study the peri-implant bacterial infections of bone and soft tissues after femur intramedullary pin implantation [53–55]. SS pins were used in a mouse bone implant infection model adopted from Bernthal et al. [56] to characterize the effect of nanocoating on implant biofilm infections in vivo.

Study of Fibrinogen and Fibronectin's Roles in Mediating Anti-biofilm Activity of Nanocoatings

We have observed that TMS nanocoatings reduce host plasma protein adsorption of both fibronectin and fibrinogen onto the implant surface. Since these proteins have been shown to play an important role in biofilm formation via facilitating the bacterial adhesion [33], we hypothesize that the ability of the TMS nanocoating to reduce fibrinogen and fibronectin adsorption onto implant surface is a driving force

underlying its inhibitory effect on biofilm formation. These studies not only can elucidate the mechanisms important for understanding how nanocoatings work, but also identify additional functional readouts such as protein adsorption to screen new materials for expanded and improved biological and clinical functions. The protein adsorption of fibrinogen, fibronectin, and albumin on SS coupons was measured by an ELISA approach [57].

Results and Discussion

Using low temperature plasma deposition technology, we fabricated nanoscale (20~30 nm) coatings of TMS or its mixture with oxygen on the surfaces of 316L SS and a Grade 5 Ti alloy of medical grade widely used in making orthopedic implants. This plasma process was performed using a DC plasma source. The silicon-containing monomer, trimethylsilane can be polymerized and deposited rapidly onto the metallic substrate surface with strong adhesion inside the plasma deposition reactor. Plasma-deposited organosilicon coatings exhibit not only a dense film as conventional plasma coatings do, but also provide a certain level of abrasion resistance for the surface of orthopedic implants due to its inorganic -Si-Si- and -Si-C-Si- backbone. The good adhesion is attributed to the formation of the -Si-O-Fe- or -Si-O-Ti- chemical bonds between the plasma-deposited layer and the oxide layer on the substrate surface. Because surface chemistry, topography, and wettability, among many other factors, affect cell attachment and interaction to the surface of biomaterials [58], we have investigated those surface properties in our preliminary studies.

Surface chemistry of coated surfaces: The surface chemistry of the coated SS and Ti substrates was analyzed with XPS. High-resolution scans of C1s were conducted for control SS, SS with TMS plasma coating, control Ti, and Ti with TMS plasma coating [49].

Compared to the uncoated SS, the surface with the TMS coating exhibited more components, with a binding energy of 284.5 eV, indicating a large amount of CH_3 formed on the surface, which could contribute to decreased protein adoption and bacterial attachment [49]. A similar phenomenon was also observed on Ti surfaces coated with a TMS coating, indicative of functional CH_3 groups generated at the surface regardless of the underlying bulk material.

Plasma nanocoatings of TMS mixed with oxygen at various ratios deposited on SS substrates were also analyzed and the elemental composition data are listed in Table 1. Increasing O_2 mass flow in the coating process resulted in decreased C percentage and elevated O while the Si percentage remained relatively stable, indicative of more Si-O formation on the coated surface, which could be one of the causes leading to reduced biofilm formation and improved osteoblast functions as described in the following subsections.

Water contact angle: As an indication of surface wettability affecting cell attachment, contact angle has been measured. The results (Fig. 1) demonstrated

Table 1 Elemental composition of SS as determined by XPS survey scan (atomic %)

Sample ID	C	Si	O	Zn	Ni	Fe	Cr	N	Mg
316L SS	27.71	1.37	47.72	0.31	0.45	15.84	4.39	0.33	1.87
TMS	50.34	25.09	24.57	0	0	0	0	0	0
TMS/O_2 1:1	33.24	24	42.76	0	0	0	0	0	0
TMS/O_2 1:2	23.13	23.49	53.38	0	0	0	0	0	0
TMS/O_2 1:4	12.07	26.61	61.32	0	0	0	0	0	0
TMS/O_2 1:8	6.87	28.09	65.04	0	0	0	0	0	0

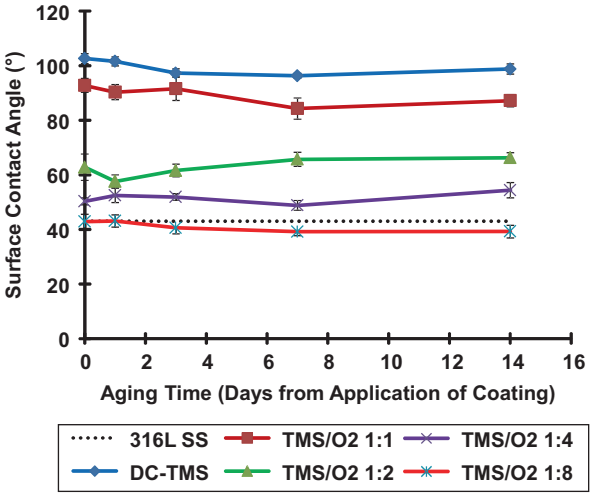

Fig. 1 Surface contact angle stability measurements over 14-day period following coating deposition

that the TMS plasma coating without oxygen rendered the surface of SS more hydrophobic (the contact angle for bare SS was about 40° as shown in the figure), whereas increasing the ratio of O_2 for the coating process tended to turn the TMS plasma coated surfaces more hydrophilic. On the other hand, the data also suggested that all the coated SS surfaces appeared to be very stable during the time period of 2 weeks after coating deposition. Similar contact angle results were also seen on Ti surfaces: 99° ± 6° for TMS coated, 43° ± 5° for TMS + O_2 (1:4), and 66° ± 7° for bare Ti (n = 5).

Plasma nanocoatings displayed strong adhesion to stainless steel surfaces: Robust adhesion of functional nanocoatings to implant surfaces is a fundamental feature required for clinical applications since premature delamination will lessen its benefit. Our initial test of a TMS coating to 2 × 2 inch SS wafers via standard ASTM D 3359 indicated that there was no coating coming off of the cross-hatched and surrounding area, indicative of strong adhesion to the underlying surface, which warrants the coating integrity on the surface of orthopedic implants during the clinical implantation procedure.

TMS coating displayed potent inhibition of staphylococcal biofilm formation: We found that there was significantly less biofilm formation on both SS and Ti

coated with TMS plasma nanocoating by *S. epidermidis* (biofilm forming RP62A strain) than uncoated controls in vitro [49], with a reduction of 99.6 ± 0.6% on SS and 99.6 ± 0.2% on Ti. Only sporadic cells or cell clusters were observed on TMS coated surfaces while multilayer biofilms were formed on uncoated surfaces [49].

Further modification of the coating process was made to incorporate oxygen into the coating to inhibit *S. aureus* biofilm formation. A variety of mixtures of TMS with oxygen at molar ratio of 1:1, 1:2, 1:4, and 1:8 were used to deposit plasma coatings on SS surfaces, and it was found the ratio of TMS to O_2 at 1:4 could generate the highest inhibition of *S. aureus* (biofilm forming NRS234 strain) [59] biofilm formation on SS surfaces while also demonstrating significant inhibition of *S. epidermidis* biofilm (Fig. 2a). Similar anti-biofilm activity was also observed on silicone substrate [57]. Very recently we have completed an immersion test using SBF to study the long-term stability of stainless steel wafers with nanocoatings (test procedure described in Subsection "Evaluation of Durability of Bioactivity and Nanocoating Integrity"). The anti-biofilm activity of the TMS/O_2 coating was well preserved (~80%) against *S. aureus* (NRS234) after 8 weeks of immersion.

Immediately upon insertion into the host, the surface of implants adsorbs plasma and extracellular proteins, such as fibrinogen and fibronectin [60]. *S. aureus* and *S. epidermidis* display a number of bacterial surface proteins that specifically bind to fibronectin and fibrinogen [61], which may mediate bacterial attachment to biomaterials [61, 62]. Interestingly, as shown in Fig. 2a, the TMS/O_2 1:4 nanocoating significantly decreased fibrinogen deposition on SS surfaces ($p < 0.04$) with a trend of decreased fibronectin binding. This finding suggests that the TMS nanocoating prevents the adhesion of proteins that favor bacterial adhesion to inhibit biofilm formation.

TMS nanocoatings displayed anti-infection efficacy: To further analyze the in vivo anti-infection efficacy of the nanocoating, TMS/O_2 1:4 coated and uncoated SS pins were dipped into 10^8 CFU of methicillin-resistant *S. aureus* (MRSA) strain NRS 384 and implanted into the femoral intramedullary canal of 12-week-old male C57BL/6 mice following a bone implant model by Bernthal et al. [56]. The mice receiving TMS/O_2 1:4 SS pins demonstrated markedly improved symptoms than mice with uncoated pins at 4 weeks with less swollen knee joints and less inflammation around the implantation sites by visual examination (Fig. 2b). The results suggested that the TMS nanocoating could reduce infection and inflammation caused by *S. aureus* infection of bone implants.

TMS nanocoatings displayed osteoconductivity for bone regeneration: Optimal bone regeneration around implants is critical for long-term success of implants. Intriguingly, we observed that when the murine osteoblast MC3T3-E1 cells were cultured on the TMS/O_2 1:4 coated SS for 7 or 14 days in vitro, they displayed significantly increased cell numbers (Fig. 3a) and alkaline phosphatase (ALP) protein levels (Fig. 3b), compared with those growing on uncoated surface. These results demonstrated that TMS/O_2 1:4 stimulated proliferation and differentiation of murine osteoblasts in vitro. Importantly, the TMS nanocoating also stimulated the gene expression of osteoblast marker bone sialoprotein (BSP) (Fig. 3c), a cell surface adhesion molecule, and induced ALP protein expression (Fig. 3d) in

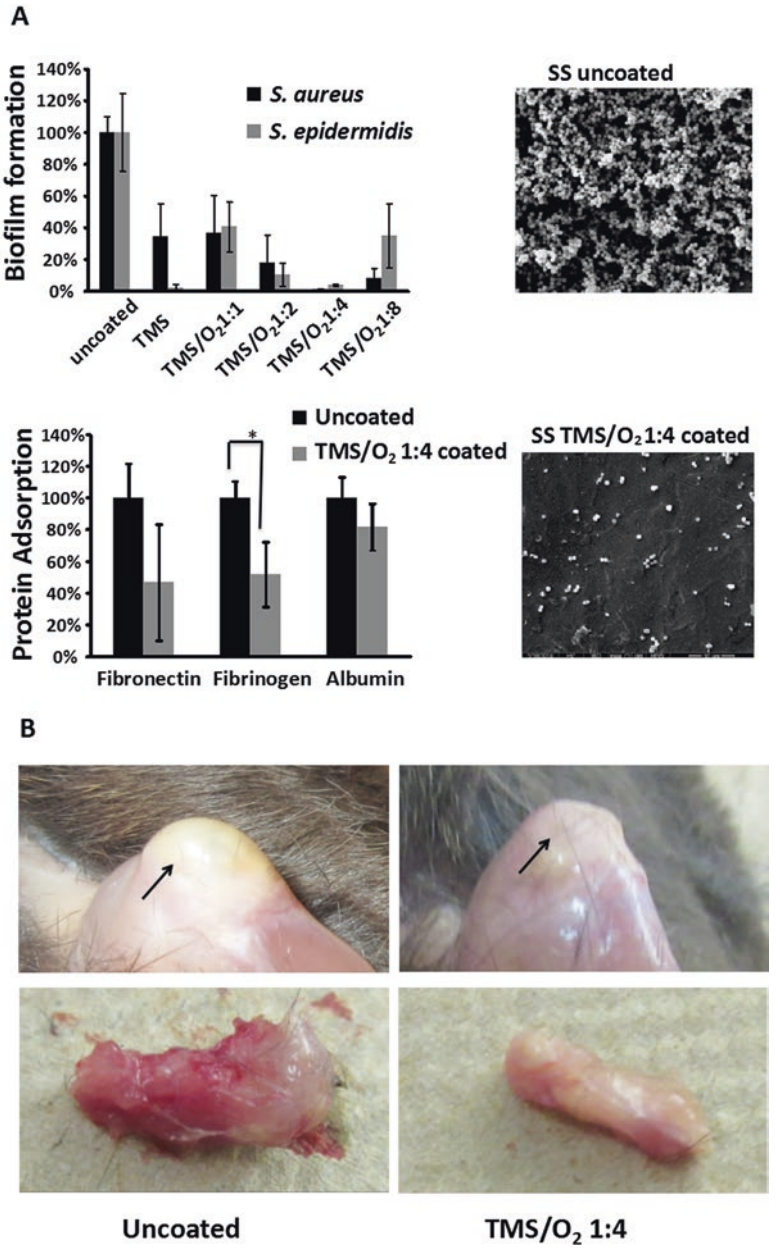

Fig. 2 Nanocoatings inhibited biofilm-related infections. (**A**) TMS/O_2 1:4 nanocoating inhibited *staphylococcal* biofilm formation and protein adsorption ($n = 3$). Student's t tests were performed. A p-value of <0.05 was considered to be statistically significant. Data was pooled from three samples and presented as mean ± standard deviation. * $p < 0.05$. (**B**) Mice implanted with MRSA-infected SS pins coated with TMS/O_2 1:4 nanocoating exhibited reduced infection and inflammation symptoms

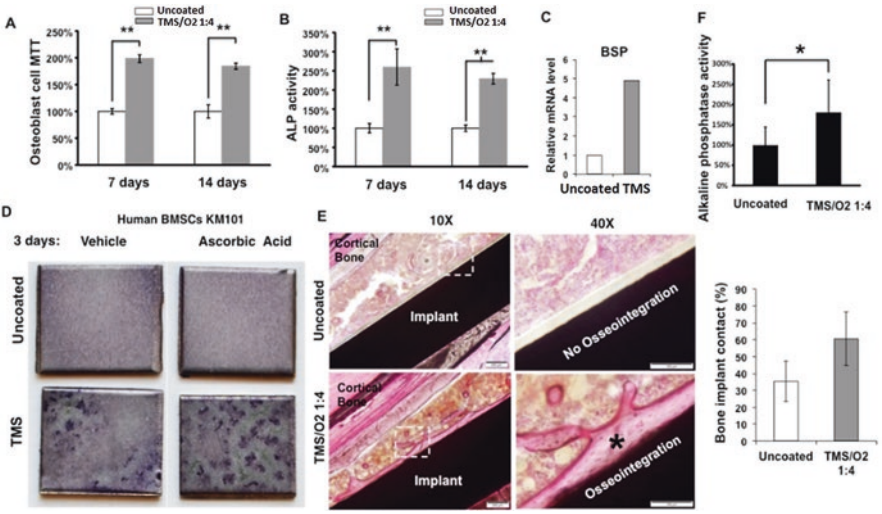

Fig. 3 TMS nanocoatings promoted osteogenesis. (**A**, **B**) TMS/O_2 1:4 coated SS surfaces promoted (**A**) murine osteoblast proliferation and (**B**) alkaline phosphatase levels, compared with uncoated surfaces ($n = 9$; $^{**}P < 0.01$). (**C**, **D**) TMS coating stimulated osteoblastic differentiation of human BMSCs, as reflected by increased human BSP mRNA levels, as shown by (**C**) qRT-PCR and (**D**) enhanced ALP staining, compared with uncoated surfaces. (**E**) H&E stain and quantification of bone-implant contact on the TMS/O_2 1:4 coated pins, compared with the uncoated pins, in vivo ($n = 3$) with $*$ indicating newly formed bone tissues. (**F**) Mice implanted with the TMS/O_2 1:4 coated pins demonstrated significantly increased serum levels of ALP activity, compared with the control mice with uncoated pins in the presence of MRSA ($n = 9$ in uncoated group, $n = 10$ in TMS/O_2 1:4 group)

human bone marrow stromal cells (hBMSCs) KM101. Taken together, these observations demonstrate that the TMS nanocoatings have a potent osteoconductive capacity for both murine and human osteoblastic cells in vitro.

Moreover, the TMS/O_2 1:4 coated SS pins, when implanted into the mouse femoral intramedullary canals, displayed markedly increased bone-implant contact (%) at 8 weeks post surgery, when compared with uncoated SS pins (Fig. 3e). Hematoxylin and Eosin (H&E) staining demonstrated that the TMS/O_2 1:4 coated SS pin surfaces were covered by well-organized bone tissues; in contrast, the infiltration of adipocytes, inflammatory cells, and fibrous tissues were often found associated with the uncoated SS (Fig. 3e). Furthermore, intriguingly, even in the presence of bacterial infection, the mice receiving TMS/O_2 1:4 pins displayed significantly increased serum levels of ALP, indicating increased bone formation in these mice, compared with those receiving uncoated pins (Fig. 3f). The former mice also displayed significantly reduced levels of bacterial infection and local tissue inflammation (Fig. 2b). Taken together, the results demonstrate the potent stimulatory effects of the TMS nanocoatings on osteogenesis both in vitro and in vivo, with or without bacterial infections.

Future research in this area will cover fundamental understanding of interaction of host and bacteria on foreign body implant, or study of mechanisms. Integration of an implant into bone, determining the long-term performance of the device, takes place largely at the interface of tissue and implant. Surface chemistry and surface topography of the implant, among other various factors, could impact the development of this interface [33]. Infections associated with orthopedic implants are manifested by bacterial colonization and biofilm formation on the implanted device and infection of the adjacent tissues. It is thus imperative to understand how implant surface chemistry and topography modulate host protein adsorption, bone cell and bacterial cell signaling. The novel dual-function nanocoating discussed here in this entry could also serve as a research tool to explore the cellular and molecular mechanisms underlying the interaction of host and bacteria on foreign body implant. By identifying critical host and bacterial genes and proteins that contribute to the dual anti-biofilm and osteoconductive properties of the nanocoatings, we would not only shed light on bone development and bacterial pathogenesis, but also identify novel therapeutic molecular targets for treating infectious and bone diseases.

Conclusions

Biofilm-inhibiting and osseointegration-promoting orthopedic implants are in urgent need to battle implant-related infections and lack of integration of implant with bone tissue. We have identified novel TMS nanocoatings with acceptable mechanical durability and unique dual properties of anti-biofilm formation and osteoconductivity, which would warrant further development of TMS coating technology for better mechanical duration, biological compatibility as well as more potent anti-biofilm and osteoconductivity. Our preliminary studies have demonstrated that $-CH_3$ and Si-O groups could be major surface factors that regulate the anti-biofilm function of the TMS nanocoatings. Further, we have found out that the molecular mechanisms underlying the dual functions of the TMS nanocoatings can be distinct from but intricately linked with and mutually beneficial to each other, thus making the optimization of the dual functions possible. Successful application of this nanocoating technology may not only lead to improved clinical efficacy, increased quality of life, and decreased health care costs to the patients, but also generate rich fundamental knowledge of the complex and highly inter-related events occurring at the implant surface after implantation.

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