REVIEW

Nanoscale Morphologies on the Surface of 3D-Printed Titanium Implants for Improved Osseointegration: A Systematic Review of the Literature

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Abstract: Three-dimensional (3D) printing is serving as the most promising approach to fabricate personalized titanium (Ti) implants for the precise treatment of complex bone defects. However, the bio-inert nature of Ti material limits its capability for rapid osseointegration and thus influences the implant lifetime in vivo. Despite the macroscale porosity for promoting osseointegration, 3D-printed Ti implant surface morphologies at the nanoscale have gained considerable attention for their potential to improve specific outcomes. To evaluate the influence of nanoscale surface morphologies on osseointegration outcomes of 3D-printed Ti implants and discuss the available strategies, we systematically searched evidence according to the PRISMA on PubMed, Embase, Web of Science, and Cochrane (until June 2022). The inclusion criteria were in vivo (animal) studies reporting the osseointegration outcomes of nanoscale morphologies on the surface of 3D-printed Ti implants. The risk of bias (RoB) was assessed using the Systematic Review Centre for Laboratory Animal Experimentation (SYRCLE's) tool. The quality of the studies was evaluated using the Animal Research: Reporting of In Vivo Experiments (ARRIVE) guidelines. (PROSPERO: CRD42022334222). Out of 119 retrieved articles, 9 studies met the inclusion criteria. The evidence suggests that irregular nano-texture, nanodots and nanotubes with a diameter of 40–105nm on the surface of porous/solid 3D-printed Ti implants result in better osseointegration and vertical bone ingrowth compared to the untreated/polished ones by significantly promoting cell adhesion, matrix mineralization, and osteogenic differentiation through increasing integrin expression. The RoB was low in 41.1% of items, unclear in 53.3%, and high in 5.6%. The quality of the studies achieved a mean score of 17.67. Our study demonstrates that nanostructures with specific controlled properties on the surface of 3D-printed Ti implants improve their osseointegration. However, given the small number of studies, the variability in experimental designs, and lack of reporting across studies, the results should be interpreted with caution.

Keywords: nano-pattern, 3D-printed, titanium implant, surface modification, osseointegration

Introduction

In the past decade, additive manufacturing, also known as three-dimensional (3D) printing, has become a promising technology for the production of orthopedic implants due to its ability to produce customized shapes for the precise treatment of complex bone defects, which are difficult to conquer with traditional methods.[1–4] Based on the computer-aided manufacturing, laser technology, numerical control technology, and polymer materials, 3D printing has shown

remarkable advantages, including single-piece customization, adjustment of mechanical strength, and high process efficiency.[5] Selective laser melting (SLM), selective laser sintering (SLS), electron beam melting (EBM), laser engineered net shaping (LENS), and direct metal laser sintering (DMLS) are the main technologies for manufacturing 3D-printed orthopedic implants, with commercially pure titanium (cp Ti) and Ti alloys (mainly Ti6Al4V) serving as the most common materials being used due to their biocompatibility, low toxicity, high corrosion resistance, high-strength-to-weight ratio, resistance to fatigue deformation and excellent mechanical properties.[6–8] However, as a bio-inert material, surface-unmodified Ti lacks biointegration capability and has a far higher elastic modulus (110–120 GPa) than that of cortical bone (10–30 GPa), which limits the long-term fixation and lifespan of Ti implants.[9]

Homogeneous interconnected porous structures within the 3D-printed implants can promote the growth of bone tissue into them, and optimize the elastic modulus by changing the porosity, hole shape, diameter, and connectivity.[10,11] It has been reported that rhombic dodecahedron- or diamond-shaped pores with a pore size of 300–600μm and 60–78% porosity can sufficiently promote bone ingrowth in 3D-printed Ti implants in preclinical studies.[12,13] Wang et al[14] and Yu et al[15] suggested that such shapes of pores in 3D-printed Ti implants were ideal choices for closely resembling cancellous bone and had a higher yield stress in bone defect animal models. Ran et al[16] and Han et al[17] found that a 3D-printed Ti scaffold with a pore size of 600 μm has more osteogenic capability than 400, 500, 700, and 800 μm in rabbit. Luan et al[12] suggested that 78% porosity had higher osteogenic capacity than 65% and 55%. In addition to micro scale design on the structures of Ti implants, micro-nano and nanoscale surface modification have been reported to further enhance osteogenesis and osseointegration by mimicking the heterogeneous structure of the natural bone tissues.[18–20] Nanostructured surface engineering to enhance implant osteointegration properties is considered one of the most promising strategies to achieve next-generation 3D-printed Ti implants.[21]

Nanoscale surface morphologies regulate osteogenic differentiation of stem cells, tissue regeneration, cell adhesion, and gene expression better than that of microscale surface structures,[22–24] for their size more closely resembles that of collagen, protein, and cell membrane receptors in the extracellular matrix (ECM).[25,26] Moreover, they help lower the contact angles at the surface, making it hydrophilic and further improving early-stage osseointegration.[27,28] Laser etching, acid etching, acid–alkali treatment, anodic oxidation, sandblasting, alkali–acid–heat treatment, and a broad range of other approaches have been explored with demonstrated ability to fabricate different forms of nanomorphology such as nanotubes, nanocolloids, nanopits, nanofibers, nanodots, nanopillars and nanogrooves.[29,30] As the nanoscale surface morphology features may further facilitate the bone growth into microscale porous structures, the past decade has witnessed a surge of investigations evaluating the effects of various 3D printing and nanostructured surface engineering technologies on osseointegration.[31–34] The results of in vitro studies have demonstrated the osteogenic properties of surface nanomorphologies on 3D-printed Ti implants and have been further validated in the in vivo studies, however, with heterogeneous results.[35–37] The relationship between surface nanoscale features and in vivo osseointegration outcomes of 3D-printed Ti implants remains challenging to interpret, owing to the highly variable study designs and the influence of multiple experimental variables on outcome measures. This review study aims to evaluate the effect of nanoscale surface morphologies on osseointegration outcomes of 3D-printed Ti implants in the preclinical studies and summary the strategies to pave the way of translating those from research to clinical application.

Methods
Systematic Literature Search
A comprehensive systematic review was performed independently following the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) guidelines (Figure 1).[38] The focused question was "Does the nanomorphology on the surface of 3D-printed Ti implants enhance the osseointegration?" and was conceived according to Participants, Interventions, Control, and Outcomes (PICO) principles,[39] as follows: (P) participant: animals received implantation; (I) intervention: 3D-printed Ti nanoscale surface modification; (C) control group: 3D-printed Ti implants without nanostructured surface modification; (O) outcome: experimental parameters related to osseointegration (ie, histological analysis, micro-CT evaluation, and biomechanical tests).
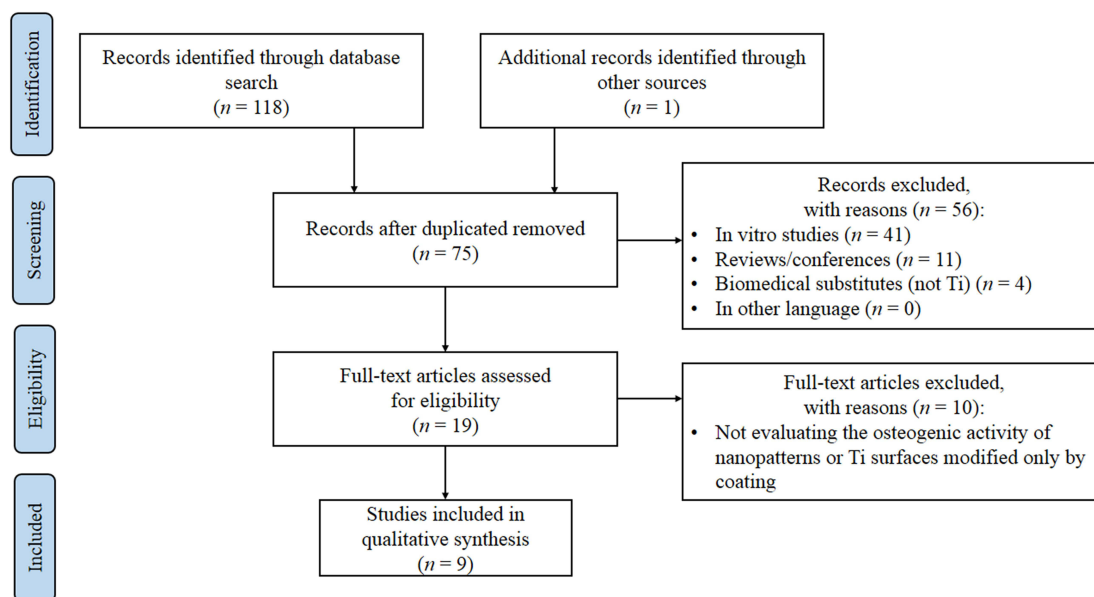
Figure 1 PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) diagram including study algorithm.

Study methods followed the Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) statement and were documented in an international prospective register of systematic reviews (PROSPERO) protocol with registration ID: CRD42022334222. To filter studies relevant to the focused question, relevant manuscripts were searched for published up to June 2022 using 4 electronic databases: PubMed, Embase, Cochrane, and Web of Science. A general search was conducted in PubMed using the following terms: ("nano" [All Fields] OR "nano scale" [All Fields] OR "nanopattern" [All Fields] OR "nanomorphology" [All Fields] OR "nanostructures" [MeSH Terms] OR "nanostructures" [All Fields]) AND ("surface" [All Fields] OR "surfacing" [All Fields] OR "surface modification" [All Fields] OR "surface engineering" [All Fields]) AND ("osseointegration" [MeSH Terms] OR "osseointegration" [All Fields] OR "bone integration" [All Fields]) AND ("titanium" [MeSH Terms] OR "titanium" [All Fields] OR "Ti" [All Fields]) AND ("implant" [All Fields] OR "scaffold" [All Fields] OR "prostheses and implants" [MeSH Terms] OR "prostheses and implants" [All Fields]) AND ("3D printing" [All Fields] OR "printing, three dimensional" [MeSH Terms] OR "3d printed" [All Fields] OR "additive manufacturing" [All Fields]). And we use keywords in combination with 'AND' or 'OR' (Boolean logic operators): (('nano' OR 'nano scale' OR 'nanopattern' OR 'nanomorphology' OR 'nanostructure') AND ('surface' OR 'surfacing' OR 'surface modification' OR 'surface engineering') AND ('osseointegration' OR 'bone integration' AND 'titanium' OR 'Ti') AND ('implant' OR 'scaffold' OR 'prothesis') AND ('3D printing' OR '3D-printing' OR '3D-printed' OR '3D printed' OR 'additive manufacturing')) to identify the relevant literature in other databases. Two authors (SY and WJ) independently inspected the titles and abstracts of the manuscripts following the eligibility criteria. Then, the full texts of the eligible manuscripts were browsed to select studies appropriate for this systematic review. The reference lists of relevant original and reviews identified in the previous step were further hand-searched. The two authors discussed the selection process until a consensus was reached. The search strategy is shown in Figure 1.

Exclusion and Inclusion Criteria

In vivo peer-reviewed studies evaluating the osseointegration outcomes of the nanostructured surface of 3D-printed Ti implants were included in this systematic review. In vivo was defined as animal studies investigating osseointegration outcomes after treatment with 3D-printed Ti implants. Each animal study was classified according to the Centre for Evidence-Based Medicine's system for assigning levels of evidence, with all studies in this review being considered basic science studies (level 5). Inclusion criteria for in vivo studies were animal studies of osseointegration outcomes after treatment with nanostructured 3D-printed Ti implants. The exclusion criteria for all studies (in vivo) were as follows: (1) articles not written in English; (2) review and expert opinion articles, conference proceedings, and presentations; (3) ex

vivo studies; and (4) studies that did not evaluate the osseointegration capacity of nanopatterns or did not modify Ti surfaces with nanopatterns. Studies were also excluded if the 3D-printed Ti implants were modified only by coating (Table S1).

Risk of Bias and Quality Assessment of the Studies Included

Assessment of the risk of bias of the selected manuscript according to the Systematic Review Centre for Laboratory Animal Experimentation (SYRCLE's tool) with a focus on ten questions to describe selection bias, performance bias, detection bias, attrition bias, reporting bias, and other bias.[40] The bias for each selected study was evaluated, where a "yes" judgment indicated a low risk of bias, and a "no" judgment indicated a high risk of bias. The judgment was "unclear" if insufficient details had been reported to properly assess the risk of bias. A low risk of bias of the study was considered if at least 7 items were assessed as "low risk" and no item was assessed as "high risk". Studies were judged as high risk of bias if at least 2 items were judged as "high risk". A moderate risk of bias was judged in other cases. Assessment of methodological quality for each study followed the Animal Research: Reporting of In Vivo Experiments (ARRIVE) guidelines.[41] Assessments were done by two independent reviewers (SY, WJ), and disagreements were resolved after consensus-oriented discussions. If disagreement occurred, the senior author (YS) was consulted.

Results

Identification and Selection of Studies

Electronic database searches identified 75 articles (Figure 1). After screening titles and abstracts for relevance, 56 articles were deemed irrelevant based on the inclusion and exclusion criteria. Out of the 19 full texts of the in vivo animal studies assessed for eligibility, 9 papers were selected and reviewed after applying the criteria. The 10 excluded articles did not evaluate the antibacterial activity of nanopatterns or involve modification of Ti surfaces with a coating. Meta-analysis was not conducted due to the scarcity and heterogeneity of the studies (Figure 1). Figure 2 shows the frequency of publications over the past decade. There has been a substantial increase in publications in recent years, reflecting the growing interest in the field of nanostructured surfaces of 3D-printed Ti implants (Figure 2).

In vivo Study Characteristics

The general characteristics of the selected in vivo studies are shown in Table 1. Four studies[42–45] used New Zealand White rabbits, and five used Sprague Dawley rats,[35–37,42,46] while athymic nude rats were used in one study.[47] Sample sizes of the included studies ranged from 10 to 48 animals,[37,47] with an unknown number used in two studies.[45,46] The distal femurs were used as the implant location in seven studies,[35–37,44–46] the top of the cranial bone in two studies,[42,47] the tibiae in one study, and the (human) mandible in the same study.[42] Follow-up periods ranged from 4 to 10 weeks. Micro-CT scan, mechanical tests, and histological analysis were performed to comprehensively assess the osseointegration outcomes. The bone volume (BV), bone volume fraction (BV/TV, %), trabecular thickness (Tb.Th, μm), trabecular
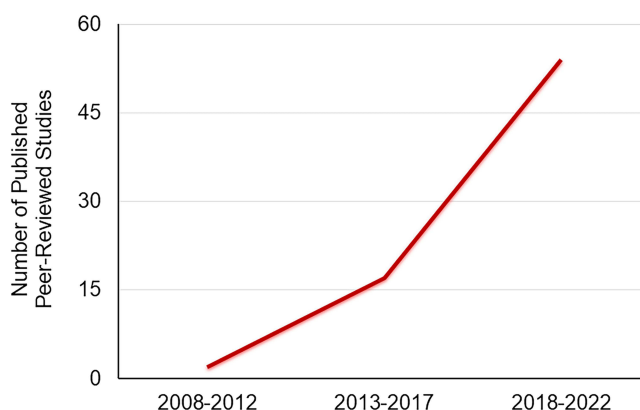

Figure 2 Frequency of studies evaluating osseointegration of the nanostructured surface of 3D-printed Ti implants per five years.

Table 1 General Characteristics of the Selected Studies

Authors (Year)	Study Animals (n, Age)	Location of Implant Placement	Study Groups	Follow-Up	Analysis Method	In vivo Osseointegration Outcomes
Ren B et al (2021)[35]	Sprague Dawley rats (27, 6 weeks)	Distal femur	Group 1: flat smooth surfaces Group 2: rougher morphology with melting traces and defects Group 3: clear smoother surfaces with micro/submicron pits Group 4: surfaces with nanotube structure	8 weeks	Micro-CT (BV/TV, Tb.Th, Tb.N, Tb.Sp) Histological analysis	Group 4 had a significantly greater BV/TV, Tb.Th and Tb.N than other groups.
Cheng A et al (2017)[47]	Athymic nude rats (48, 8 weeks)	Top of cranial bone	Group 1: solid implants with macro-, micro- and nano-features Group 2: porous implants with macro-, micro- and nano-features Group 3: porous implants with macro-, micro- and nano-features and DBX	10 weeks	Micro-CT (BIC, bone volume into implant pores) Biomechanical Test (Pull-out test) Histological analysis (BIC)	BIC and pull-out values were higher for Group 2 implants compared to Group 1.
Bandyopadhyay A et al (2017)[36]	Sprague Dawley rats (12, N/A)	Distal femur	Group 1: dense TiGroup 2: porous LENS™ Ti Group 3: porous LENS™ Ti implants with TiO$_2$ nanotubes	10 weeks	Micro-CT Biomechanical Test (Push-out force) Histological analysis SEM characterization (interfacial bonding)	Group 3 had better signs of bonding and bone tissue integration with negligible gap than other groups. The interfacial shear modulus for porous samples is higher than Group 1.

(*Continued*)

Table 1 (Continued).

Authors (Year)	Study Animals (n, Age)	Location of Implant Placement	Study Groups	Follow-Up	Analysis Method	In vivo Osseointegration Outcomes
Cohen DJ et al (2016)[42]	Sprague Dawley rats (12, 8 weeks)	Top of cranial bone	Group 1: smooth implantsGroup 2: rough implants combined micro- and nano-roughness Group 3: rough implants combined roughness and DBX	10 weeks	Micro-CT (BIC, Bone Ingrowth) Biomechanical Test (Pull-out force, Modulus) Histological analysis (BIC, Bone Ingrowth)	Group 2 had higher BIC and pull-out force than Group 1, while Group 3 had the highest outcomes in all groups.
	New Zealand White rabbits (30, N/A)	Tibia	Group 4: rough implants combined micro- and nano-roughness	6 weeks	Micro-CT (BIC) Histological analysis	Group 4 had fully formed bone inside and outside the implants.
	Human (2, N/A)	Mandible	Group 5: rough implants combined micro- and nano-roughness	8 months	X-ray CT	Implant was osseointegrated with continued functional loading.
Hyzy SL et al (2016)[43]	New Zealand White rabbits (16, N/A)	Distal femur	Group 1: implants manufactured by a traditional CNC manufacturing process without distinct nanofeatures Group 2: implants manufactured via laser sintering with nanostructures	6 weeks	Micro-CT (BIC) Biomechanical Test (Pull-out test) Histological analysis (BIC)	Osseointegration was achieved for both groups but Group 2 had higher BIC than Group 1. However, no significant differences were found in pull-out test.
Wang H et al (2018)[37]	Sprague Dawley rats (10, N/A)	Distal femoral metaphysis	Group 1: smooth implants with micron-scale surface topography Group 2: implants with nodular nanostructures	4 weeks	Histological analysis (BIC)	Group 2 had more new-formed bone and higher BIC than Group 1.
Cohen DJ et al (2017)[44]	New Zealand white rabbits (20, 13–16 weeks)	Distal femur	Group 1: solid implants with nanopatterns Group 2: Porous implants with nanopatterns	10 weeks	Micro-CT (Bone Volume) Biomechanical Test (Pull-out force) Histological analysis (BIC, bone area)	An interconnected network of trabecular-like bone was observed around and penetrating through porous implants in Group 2.

Mitra I et al (2021)[45]	Sprague-Dawley rats (N/A, N/A)	Distal femur	Group 1: microporous CpTi implants (CpTi-P) Group 2: Ti implants with nanotubes (TNT)	5 weeks	Micro-CT Histological analysis (OS/BS %)	In comparison to Group 2, Group 4 and Group 7 show a higher presence of osteoid. Group 7 displayed decreased gap width compared to Group 2, with Group 9 exhibiting a higher OS/BS % than Group 7 and 8.
	New Zealand White rabbits (N/A, N/A)	Distal femur	Group 3: microporous Ti implants with nanotubes (TNT-P) Group 4: Ti-10%Ta implants with nanotubes (10Ta-NT) Group 5: microporous Ti-10%Ta implants (10Ta-P) Group 6: microporous Ti-10%Ta implants with nanotubes (10Ta-P-NT) Group 7: Ti-25%Ta implants with nanotubes (25Ta-NT) Group 8: microporous Ti-25%Ta implants (25Ta-P) Group 9: microporous Ti-25%Ta implants with nanotubes (25Ta-P-NT) Group 10: microporous Ti-100% Ta implants (100Ta-P)	7 weeks	Histological analysis (%Bone apposition, %Trabecular bone, Blood vessels, Bone remodeling)	Group 1 shows mildly dense while Group 8 and 9 exhibit moderately dense bony proliferation of woven bone. The percentage of trabecular bone and the total number of blood vessels is higher around the implant-bone interface for Group 9 compared to Group 1 and 10.
Bose S et al (2018)[46]	Sprague Dawley rats (N/A, N/A)	Distal femur	Group 1: LENS porous Ti Group 2: LENS porous Ti with nanotubes (Ti-NT) Group 3: LENS porous Ti with nanotubes and CaP coating morphology (Ti-NT-CaP) Group 4: LENS porous Ti with nanotubes and Sr-Si-CaP coating morphology (Ti-NT-CaP-Sr-Si)	10 weeks	Micro-CT Biomechanical Test (Modulus) Histological analysis (total osteoid formation, total bone formation) SEM characterization (interfacial bonding)	Group 2 has higher total osteoid formation and total bone formation and less gaps at the interface of the bone tissue and implants than Group 1. And Group 4 has the best results of the four groups.

Abbreviations: Ti, Titanium; cp Ti, Commercially pure titanium; CT, Computed tomography; BV/TV, Bone volume fraction; Tb.Th, Trabecular thickness; Tb.N, Trabecular number; Tb.Sp, Trabecular spacing; BIC, Bone to implant contact; SEM, Scanning electron microscopy; LENS™, Laser engineered net shaping; TiO_2, Titanium dioxide; CNC, Computer numerical control; DBX, Demineralized bone matrix putty; OS/BS, Osteoid surface/bone surface.

number (Tb.N, 1/mm), and trabecular spacing (Tb.Sp, μm) were analyzed by micro-CT analysis. Histological analysis included bone-to-implant contact (BIC), total osteoid formation, and total bone formation. Biomechanical tests included pull-out tests and modulus values as measures of implant stability. SEM characterizations were performed to show the interfacial bonding of the host tissue and the implants.

The implant-related characteristics of the selected studies are presented in Table 2. The number of implants applied per study ranged from 10 to 48. LENS™ had been utilized to develop the implants in two studies,[36,46] SLS had been used in five studies,[37,42–44,47] LENS™ had been used in three studies,[36,45,46] and EBM was used in one study to prepare Ti-6Al-4V implants.[35] The implant shape was rod-shaped in six studies,[35–37,44–46] cuboidal in one study,[47] disk-shaped in two studies,[42,43] and one study[42] created custom wrap implants for rabbit models and patients. The diameter and length of the implants ranged from 2.3 to 6.0 mm and 3.0 to 10.0 mm, respectively. The porosity of the 3D-printed implants ranged from 25% to 68%, with four studies did not mention the porosity.[35,37,42,43] Three studies used anodic oxidation to generate implants with porosity based on micro-/nano-surface roughness,[36,45,46] four studies produced nanoscale surface morphology via grit blasting and acid etching,[42–44,47] one study introduced an ultrasonic-assisted acid etching process,[35] and one study developed an alkali-heat treatment to fabricate nano-topography.[37] Four studies formed nanotubes with a diameter ranging from 40 to 105 nm on the surface of 3D-printed Ti implants,[35,36,45,46] four studies[42–44,47] constructed nano-texture on the surface and samples exhibited nodular nanostructures in one study.[37]

Risk of Bias and Quality Assessment of the Studies Included

The included studies presented heterogeneous levels of risk of bias, which are presented in Figure 3. Three studies reported that animals were randomly allocated to different groups during the experiment.[44,45,47] Three included studies were evaluated as having unclear baseline characteristics.[42,45,46] One study reported the labeled cages and it was unclear how the cages were labeled and how the allocation sequence was concealed in the other studies.[46] Three studies[36,44,47] clearly explained the random animal housing, while others were unclear. The risk of detection bias was low in one study.[42] One study reported that the outcome assessors were blinded, while other studies had a moderate risk of blinding detection bias.[42] All the selected studies were assessed as having a moderate risk of blinding performance bias and a low risk of reporting and other biases. Attrition bias was described as low risk in six studies[36,37,42,44–46] and high in three studies.[35,43,47] All of the studies had a moderate probability of RoB (n = 9). The risk of bias was low in 41.1% of items, unclear in 53.3%, and high in 5.6%. Assessment of methodological quality for each study was summarized in Table S2. The quality of the selected studies (ARRIVE [Animal Research: Reporting of In Vivo Experiments] guidelines) achieved a mean score of 17.67 and only two studies mentioned that they followed the ARRIVE guidelines.[44,47]

Discussion

Modern manufacturing technology constantly evolves in biomedical implant design, aiming to control what happens at the bone-implant interface. Altering surface morphology, plasma-sprayed coatings, and the construction of a porous structure through various manufacturing methods have been shown to influence the bone-implant interface in animal, preclinical and clinical studies.[48,49] 3D printing technology represents an alternative method of implant production where anatomical shapes can be produced that would be considered too complex or costly to achieve through traditional methods.[21] In terms of microstructure, 3D printing technology can precisely control the Young's modulus of the implants to match natural bone tissues by designing pores that can effectively reduce the stress-shielding effect of the implants and reduce the incidence of peri-implant osteolysis.[50] In terms of macrostructure, 3D printing technology can efficiently print the implant in a complex shape according to the patient's personalized anatomical characteristics and clinical needs through CT images to adapt the mechanical properties and custom shapes of the implants to the natural bone tissue.[6] Compared with traditional Ti implants, microscale porous structure can promote the early vascularization of 3D-printed Ti implants and improve the osteointegration.[51,52] The 3D printing technology has shown remarkable advantages in terms of free design, single-piece customization, and high process efficiency. These features allow coping with the urgent biomedical field needs, thus have attracted the interests of researchers and significantly stimulated the clinical applications of 3D printing in recent years.[5,35] Ti is currently the most popular material for 3D printing of orthopedic implants because of its advantages of excellent mechanical strength, biocompatibility and corrosion resistance in vivo.[53] However,

Table 2 Implant-Related Characteristics of the Selected Studies

Authors (Year)	Implants Number (n)	Implant Material	Implant Manufacturing	Surface Manufacturing	Implant Dimensions (Ø × L, mm)	Ti Implant Shape	3D-Printed Porous Structures	Surface Nanopatterns	Nanopattern Dimensions (Ø × L, nm)	In vivo Osteogenic Activities	Mechanisms
Ren B et al (2021)[35]	27	Ti-6Al-4V	EBM	Ultrasonic-assisted acid etching	2.5 × 3.0	Rods	Solid	Nanotubes	Ø 40	3D-printed porous implants with nanotubes on surface had more volume of new bone and better anodization and direct bone connection compared to others.	N/A
Cheng A et al (2017)[47]	48	Ti-6Al-4V	SLS	Grit blasting and acid etching	3.5 (width) × 5.0 (length) × 2.0 (height)	Cuboids	Shape: irregular Ø: N/A Porosity: 67±3%	Nano-texture (random)	N/A	The porous implants had the ability to not only osseointegrate, but also promote the vertical bone growth.	N/A
Bandyopadhyay A et al (2017)[36]	12	Ti	LENS™	Anodic oxidation	2.3 × 5.0~5.5	Rods	Shape: irregular Ø: N/A 200–300µm Porosity: 25%	Nanotubes	105±30 × 375 ±35	The porous implants showed better bone-tissue integration compared to the dense samples while the porous implants with nanotubes on surface showed better bone tissue integration than the other implants.	Nanotubes results in higher surface area making the surface contact angle low and the surface more hydrophilic to improve cell adhesion and biocompatibility.
Cohen DJ et al (2016)[42]	12 (rats)	Ti-6Al-4V	SLS	Grit blasting and acid etching	Ø 5.0	Disks	Shape: N/A Ø: N/A Porosity: N/A	Nano-texture (random)	N/A	Implant surfaces with micro-/nano-roughness enhanced osseointegration in vivo compared to smooth surfaces.	N/A
	30 (rabbits)				Customized	Custom wrap implants			N/A		
	2 (human)				Customized	Custom wrap implants			N/A		
Hyzy SL et al (2016)[43]	32	Ti-6Al-4V	SLS	Grit blasting and acid etching	3.7 × 8.0	Disks	Solid	Nano-texture (random)	N/A	Bone remodeling of Group 2 increased during the osseointegration process as compared to that of Group 1.	Integrin α2β1 mediates cell response to surfaces at the nanoscale, promoting osteoblast maturation and differentiation

(Continued)

Table 2 (Continued).

Authors (Year)	Implants Number (n)	Implant Material	Implant Manufacturing	Surface Manufacturing	Implant Dimensions (Ø × L, mm)	Ti Implant Shape	3D-Printed Porous Structures	Surface Nanopatterns	Nanopattern Dimensions (Ø × L, nm)	In vivo Osteogenic Activities	Mechanisms
Wang H et al (2018)[37]	10	Ti-6Al-4V	SLS	Alkali-heat treatment	2.0 × 3.0	Rods	Solid	Nodular nanostructures (nanodots)	N/A	Nano-3D implants with superior biocompatible and rapid vivo osseointegration ability enhanced osseointegration at the interface of bone and implants	Nodular nanostructure on 3D-printed implant surface significantly improves the hydrophilic property.
Cohen DJ et al (2017)[44]	19	Ti-6Al-4V	SLS	Grit blasting and acid etching	3.8 × 8.0	Rods	Shape: trabeculae-liked Ø: 461–872μm Porosity: 68.6% ± 0.8%	Nano-texture (random)	N/A	Implants with trabecular bone-inspired porosity can achieve superior osseointegration to solid implants	N/A
Mitra I et al (2021)[45]	2 implants per rat	Ti and Ti-Ta alloy	LENS™	Anodic oxidation	3.0 × 5.0	Rods	Shape: N/A Ø: N/A Porosity: N/A	Nanotubes	Group 4: Ø 88 ± 5 Group 7: Ø 67 ± 12	Nanoscale design elements at the surface of a porous Ti-Ta alloys implants can also be expected to enhance migration of cells, adhesion, and influence overall bone remodeling	N/A
	3 implants per rabbit				6.0 × 10.0	Rods					
Bose S et al (2018)[46]	5 implants per group	Ti	LENS™	Anodic oxidation	2.3 × 5.0–5.5	Rods	Shape: N/A Ø: N/A Porosity: 25%	Nanotubes	105±30 × 375 ±35	Porous Ti implants with TiO$_2$ nanotubes induced early-stage osteogenesis while the addition of calcium phosphate coating further enhanced defect healing and mechanical interlocking at the interface.	Nanotubes on 3D-printed implant surface facilitate enhanced osteoblast cell attachment and differentiation and the higher surface to volume ratio helps in making the surface hydrophilic.

Abbreviations: Ti, Titanium; 3D, Three dimensional; SLS, Selective laser sintering; EBM, Electron beam melting; LENS, Laser engineered net shaping; TiO$_2$, Titanium dioxide; Ta, Tantalum.

Authors (Year)	Ren B et al., 2021[35]	Cheng A et al., 2017[47]	Bandyopa dhyay A et al., 2017[36]	Cohen DJ et al., 2016[42]	Hyzy SL et al., 2016[43]	Wang H et al., 2018[37]	Cohen DJ et al., 2017[44]	Mitra, I et al., 2021[45]	Bose S et al., 2018[46]
Selection bias (Sequence generation)	Yellow	Green	Yellow	Yellow	Yellow	Yellow	Green	Green	Yellow
Selection bias (Baseline characteristics)	Green	Green	Green	Yellow	Green	Green	Yellow	Yellow	Yellow
Selection bias (Allocation concealment)	Yellow	Yellow	Yellow	Yellow	Yellow	Yellow	Yellow	Yellow	Red
Performance bias (Random housing)	Yellow	Green	Yellow	Yellow	Yellow	Yellow	Green	Yellow	Yellow
Performance bias (Blinding)	Yellow	Yellow	Yellow	Yellow	Yellow	Yellow	Yellow	Yellow	Yellow
Detection bias (Random outcome assessment)	Yellow	Yellow	Yellow	Green	Yellow	Yellow	Yellow	Yellow	Yellow
Detection bias (Blinding)	Yellow	Yellow	Yellow	Red	Yellow	Yellow	Yellow	Yellow	Yellow
Detection bias (Incomplete outcome data)	Red	Green	Green	Red	Green	Green	Green	Green	Green
Reporting bias (Selective reporting)	Green	Green	Green	Green	Green	Green	Green	Green	Green
Other sources of bias	Green	Green	Green	Green	Green	Green	Green	Green	Green

Figure 3 Risk of bias and quality assessment of the included studies based on SYRCLE's (Systematic Review Centre for Laboratory Animal Experimentation) risk of bias tool. Green indicates low risk of bias; yellow indicates unclear; and red indicates high risk of bias.

untreated titanium alloy implants always possess a bioinert surface that fails to simulate the structure of cortical bone and cancellous bone in real bone tissue and prevents reactions between the organism and implants, so surface modification technologies on 3D-printed Ti implants are essential for further osteointegration.[50]

The surface topographies of traditional Ti implants have been modified on the micro-nano scale and nanoscale, which play a positive role in osteointegration.[18,54–56] Over the past six decades, the development of surface modification of orthopedic Ti implants can be classified into four main stages. The surface technologies of implants have progressed from bio-inert surfaces to biocompatible Ti surfaces, to bioactive surfaces, to the most recent and probable future generation of biomimetic engineered, nanoscale surfaces such as Ti surface treated with Ti oxide (TiO_2) nanotube arrays. Now latest studies are approaching the stage of the fourth generation with recent remarkable advancements in cell biology, nanomedicine, material science, nanotechnology, and additive manufacturing. A broad range of surface modification techniques such as acid etching, anodic oxidation, sandblasting, and heat treatments have been explored with demonstrated ability to fabricate different forms of nanomorphology such as nanotubes, nanogrooves, nanopits, nanopillars, nanocolloids, and nanofibers. Numerous studies have confirmed greater bone-implant contact area and new bone formation on nanotubes on the surface of Ti implants.[57] Nanogrooved patterns of 50μm in parallel or radial arrangements on Ti implants have been proved to enhance the migration and proliferation of osteoblasts[58] and demonstrated superior bone regeneration than in the nonpatterned implants in vivo studies.[59] Nanopits of 30nm exhibited a more branched cell morphology and promoted early osteoblastic differentiation on Ti surfaces.[60] Sjöström et al found that cells on nanopillars of 15nm on the Ti surface showed a trend toward more large and super-mature osteogenic focal adhesions than those on the polished Ti surface and cells on the nanoscale surface had longer adhesions and produced larger osteocalcin deposits.[61] Ning et al suggested that growing nanorods with length and diameter of 100 and 20nm and a medium density on the surface of traditional Ti implants was an efficient method for promoting bone formation and osteointegration in rabbit tibia.[62] Recent in vitro and in vivo studies have focused on the osteointegration outcomes of 3D-printed Ti implants with nanomorphology like nanotubes, nanodots and nanotexture, and future preclinical studies should pay attention to other nanomorphology such as nanopits, nanogrooves, nanorods, and nanofibers. To summarize,

nanostructured surface topographies modified at the nanoscale positively contribute to the early osteoblastic differentiation on the surface of Ti in vitro, providing a novel way to enhance osteointegration of the 3D-printed Ti implants.

Numerous engineering technologies of surface nanopatterns on Ti implants have been developed during the past decade, which can be divided into physical surface modification, chemical surface modification and biological surface modification (Figure 4). Physical surface modification technologies depend on lasers, ultrasonics, high-energy particles, and magnetic fields to modify Ti materials' surface morphology and ultrastructure. These methods, such as laser surface engineering (LSE), arc ion plating (AIP), sandblasting, and ultrasonic nanotechnology, are commonly used to establish various surface topographies on the surface of implants and improve their resistance to wear, oxidation, and corrosion. The main advantage of LSE entails the capability of hierarchically controlling the chemical composition and the surface texture (pits, grooves, ablation tracks, ripples, pillars, columns) using different laser-based approaches and techniques such as: laser ablation, laser induced periodic surface structures (LIPSSs), laser melting, laser cladding, laser engineered net shaping (LENS™), direct laser interference patterning (DLIP), and matrix assisted pulsed evaporation (MAPLE).[63–65] However, physical surface modification requires more advanced equipment than chemical modification, and the resulting bonding force is weaker compared to chemical bonds. In contrast to physical methods, chemical surface modification technologies alter the chemical properties of implants by exposing them to a chemical solution or gas that bonds with bioactive substances. Chemical surface modification technologies include anodic oxidation, micro-arc oxidation (MAO), electrophoretic deposition, chemical vapor deposition, acid/alkali etching, and atomic layer deposition. In addition, implants with complex shapes always adopt chemical surface modification technologies and have excellent prospects of application in bone regeneration on the implant surface (contact osteogenesis) of 3D-printed implants. After processing Ti structural materials into the required implant design, surface modification is carried out by combining sandblasting and acid etching to form sandblasted, large-grit, and acid-etched surfaces, which commercially use the terminology SLA. Abrasive particles (110–250 μm in size) such as aluminum oxide (Al_2O_3), silicon oxide (SiO_2) or TiO_2 are used in sandblasting.[66,67] Hydrochloric acid (HCl), hydrofluoric acid (HF), nitric acid (HNO_3), and sulfuric acid (H_2SO_4) are the common acidic substances for acid
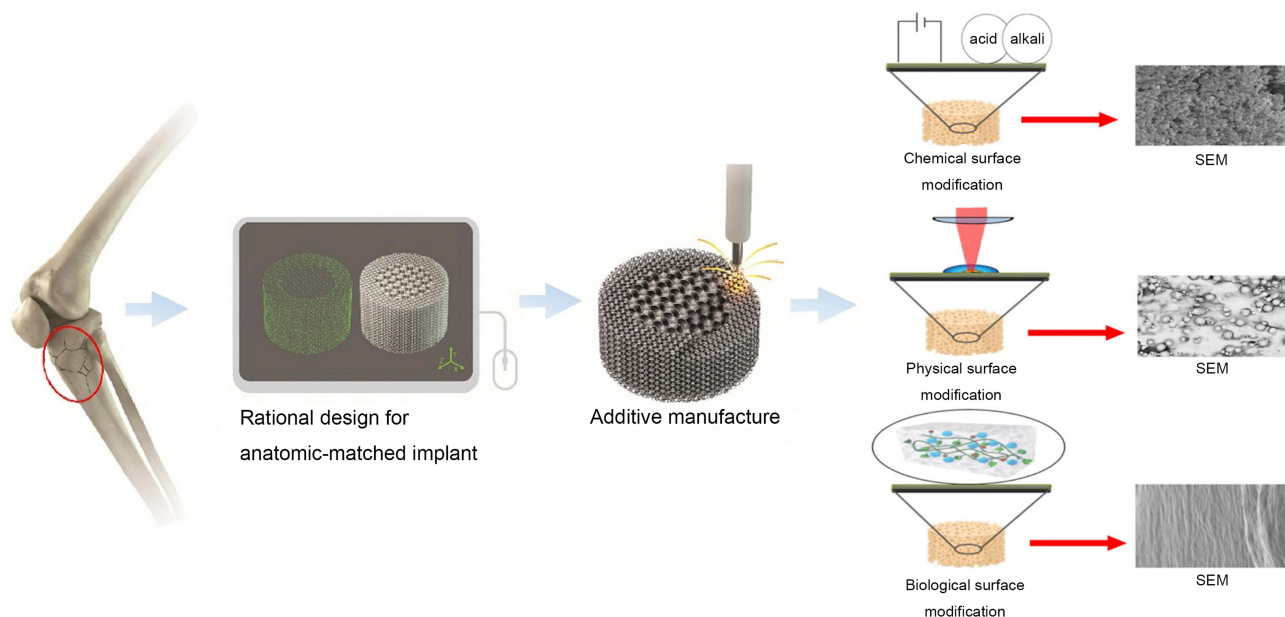

Figure 4 3D-printed Ti surface modification technologies: physical, chemical, and biological surface modification technologies. Reprinted from *J Mech Behav Biomed Mater*, Vol 105, Li J, Cui X, Hooper et al, Rational Design, Bio-functionalization and Biological Performance of Hybrid Additive Manufactured Titanium Implants for Orthopaedic Applications: A review, 103,671, Copyright © 2020, with permission from Elsevier;.[72] Reprinted from *Applied Surface Science*, Vol 480, Surmeneva M, Lapanje A, Chudinova et al, Decreased Bacterial Colonization of Additively Manufactured Ti6Al4V Metallic Scaffolds with Immobilized Silver and Calcium Phosphate Nanoparticles, 822–829, Copyright © 2019, with permission from Elsevier.[73] Reprinted from Sheng X, Wang A, Wang Z, Liu H, Wang J, Li C. Advanced Surface Modification for 3D-Printed Titanium Alloy Implant Interface Functionalization. *Front Bioeng Biotechnol*. 2022;10:850,110. Creative Commons;[50] Reprinted from Soyama H, Takeo F. Effect of Various Peening Methods on the Fatigue Properties of Titanium Alloy Ti6Al4V Manufactured by Direct Metal Laser Sintering and Electron Beam Melting. *Materials (Basel)*. 2020;13(10). Creative Commons;[74] Reprinted from Mieszkowska A, Beaumont H, Martocq et al. Phenolic-Enriched Collagen Fibrillar Coatings on Titanium Alloy to Promote Osteogenic Differentiation and Reduce Inflammation. *Int J Mol Sci*. 2020;21(17). Creative Commons.[75]

etching, which can remove entrapped abrasive particles on the air-braided Ti surfaces followed by the dissolution of the Ti oxide film and micro-scale bulk layer in the Ti subsurface.[34] Biological surface modification is a novel technology that constructs biofunctional coatings to fabricate bio-functionalized Ti-based implants, including biopolymers, growth factors, extracellular matrix proteins, peptides, and drugs on the surface of implants, which could enhance and maintain osseointegration and bioactivity of Ti alloy implants.[68–71]

3D-printed Ti implants constructed with nano-surface roughness significantly promote cell adhesion, matrix mineralization, and osteogenic differentiation, building a suitable microenvironment for cell growth.[76] The protein adsorption and cell adhesion experiments confirmed that the micro-/nano-topography notably enhanced porous Ti's protein binding capacity and promoted MSCs adhesion on the surface.[43,77] More importantly, cell differentiation experiments have shown that MSCs on the 3D-printed Ti implants with nanostructured surfaces elevated the osteoblastic gene expressions like BMP-2, BMP-4, RUNX2, osteocalcin, and vascular endothelial growth factor (VEGF), proving that nanoscale surfaces could enhance growth factor production and osteoblastic differentiation of MSCs.[43,76,78] The rapid recruitment of osteoblasts is a necessary prerequisite for the effective repair of bone defects.[79] Nanoscale roughness on the surface of 3D-printed Ti implants could improve the differentiation and maturation of osteoblasts and promote matrix mineralization, which suggested the enhanced performance of these surfaces for increasing osseointegration. However, studies on the surface modification of 3D-printed Ti implants and the effects of surface characteristics on osteogenesis in vivo are still needed for the next generation of Ti implants with improved biological performances.

Porosity in 3D-printed Ti implants helps in inducing surface roughness which results in higher surface area and promotes osteogenesis by the new bone formation and vascular ingrowth between the implant surface and the living tissue, while better interfacial bonding was seen in surface-modified porous samples with nanostructures.[71,78,80] Five of the selected studies reported that better interfacial bonding and more bone ingrowth were seen in surface-modified nanomorphology samples with porous structures.[36,44–47] Three studies reported that nanoscale design elements at the surface of porous Ti and its alloy implants could also be expected to enhance interfacial shear modulus, migration of cells, adhesion, and influence overall bone remodeling.[36,44,46] Bandyopadhyay et al[36] compared to the dense implants, 3D-printed porous samples showed better bone ingrowth, bonding of porous implants with the tissue, and new osteoid formation in the high-resolution CT scan images and histological evaluation after 4 weeks (Figure 5A). They observed almost complete bone regeneration with no gaps or incomplete bonding after 10 weeks in porous implants, which confirmed enhanced early-stage osseointegration. Cohen et al[44] suggested that 3D-printed Ti-6Al-4V implants with trabecular bone-inspired porosity and nanorough showed more bone ingrowth and deposition in the apical portions of implants in both groups, as observed via Micro-CT and histology, suggesting that mechanical strength is enhanced in porous areas. Modifying the porous implant surface topography at a nanoscale level could further facilitate the process of early-stage osseointegration. Specifically, in the primary outcomes of the six included studies,[35–37,42,45,46] the histological analysis, micro-CT evaluation, or biomechanical tests results of the nanostructured surfaces of 3D-printed Ti implants were significantly higher than the smooth surfaces, meaning better biocompatibility and stronger osteoconductive and osseointegration capabilities of the Ti nanostructured surface (Figure 5B). 3D-printed Ti implants with periodic surface nanopatterns dominated by ordered nanotubes were shown to enhance the early stage osseointegration and improve mechanical interlocking between the host bone and the implant compared to dense and pure porous Ti implants.[35,36,45,46] In the qualitative histology and Micro-CT scan, Ren et al[35] and Mitra et al[45] proved that nanotube samples had a higher value at all time domains in trabecular formation, and Bose et al[46] found that porous Ti rods with nanotube film had higher total osteoid formation and total bone formation than pure porous Ti rods (Figure 5C). Bandyopadhyay et al[36] and Bose et al[46] obtained the SEM images of the stained samples and found that the gaps reduced as they moved from dense Ti samples towards the porous samples and were significantly reduced for porous Ti implants with nanotubes, suggesting a significant improvement of osseointegration across the bone-implant interface (Figure 6). 3D-printed Ti implants with through-pores and non-periodic micro/nanoscale surface texture showed enhanced early-stage osseointegration in rat and rabbit models, as evidenced by 3D analysis of osseointegration and new bone infiltration into the pores and increased pull-out values.[37,42–44,47] All evidence suggests that irregular nano-texture, nanodots and nanotubes with a diameter of 40–105nm on the surface of porous/solid 3D-printed Ti implants result in better osseointegration and vertical bone ingrowth compared to the untreated/polished ones.
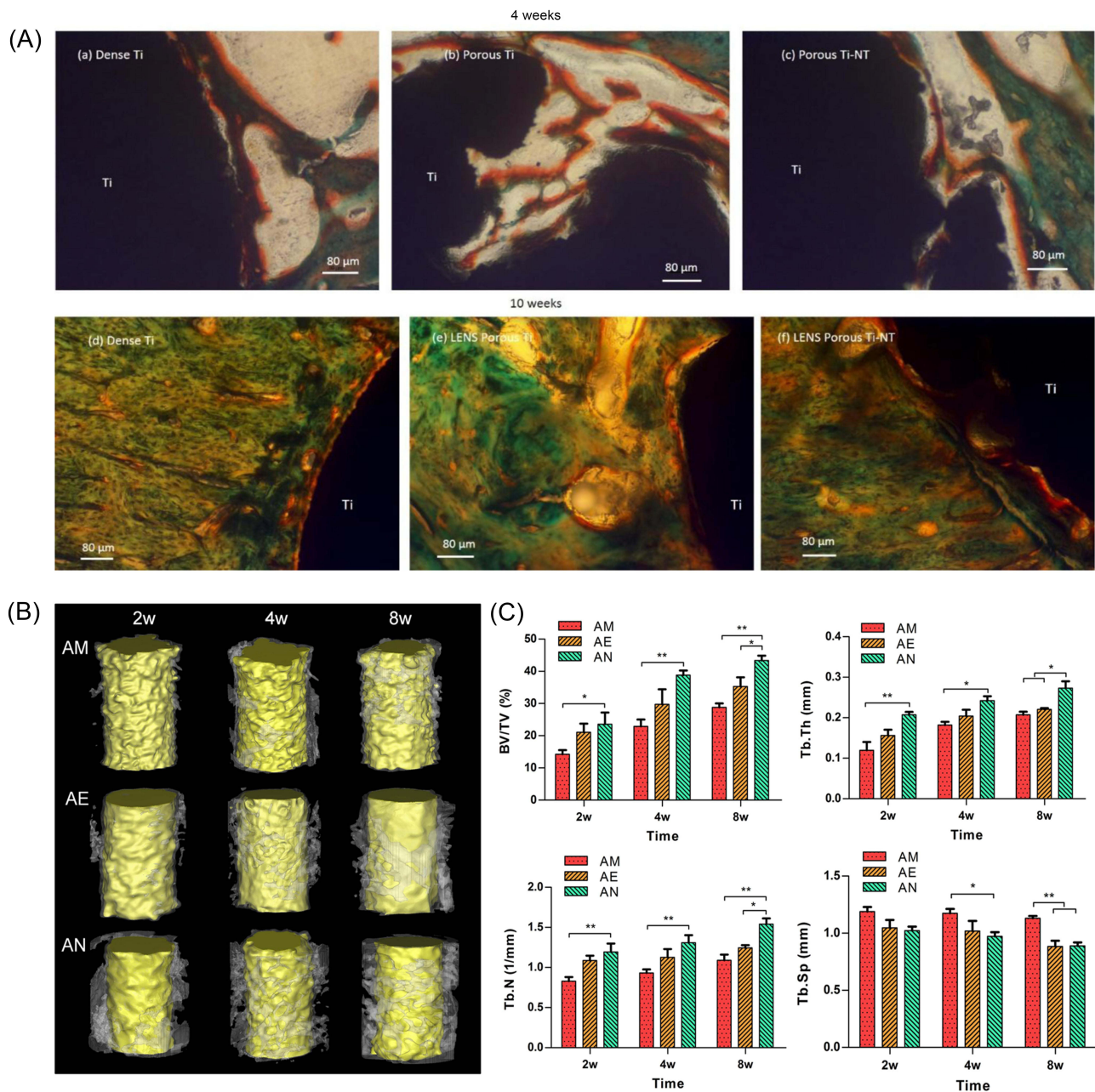
Figure 5 Evidence of 3D implants with nanoscale surface promoting osseointegration. (**A**) Histology images after 4 weeks (a, b, c) and 10 weeks (d, e, f) where signs of osteoid like new bone formation could be seen in Orange/ red color; Reprinted from *Ann Biomed Eng*, Vol 45(1), Bandyopadhyay A, Shivaram A, Tarafder et al, In Vivo Response of Laser Processed Porous Titanium Implants for Load-Bearing Implants, 249–260, Copyright © 2017, with permission from SNCSC.[36] (**B**) Micro-CT reconstructed 3D models at 2, 4, and 8 weeks; (**C**) Quantitative analysis of Micro-CT: bone volume/tissue volume (BV/TV), trabecular thickness (Tb.Th), trabecular number (Tb.N) and trabecular separation (Tb. Sp) (*p < 5%, **p < 1%). Reprinted from *Mater Sci Eng C*, Vol 118, Ren B, Wan Y, Liu et al, Improved osseointegration of 3D printed Ti-6Al-4V implant with a hierarchical micro/nano surface topography: An in vitro and in vivo study, 111,505, Copyright © 2021, with permission from Elsevier.[35]

In addition, methodological and statistical heterogeneity also exists in designing the trial protocol and response evaluation, which may eventually affect the accuracy of the analysis result.[81] No studies qualitatively compared the characteristics of different additive manufacturing technologies. Bone remodeling involves the removal of mineralized bone by osteoclasts followed by the formation of the bone matrix through the osteoblasts that subsequently become mineralized, which starts as early as 3 weeks after implantation and continues at a substantial level up to 12 weeks.[82] It is worth noting that the nanostructured surface engineering to 3D-printed Ti implant surfaces appeared to accelerate early-stage osseointegration in 10 weeks, while it has to be clarified in studies that include more extended observational periods
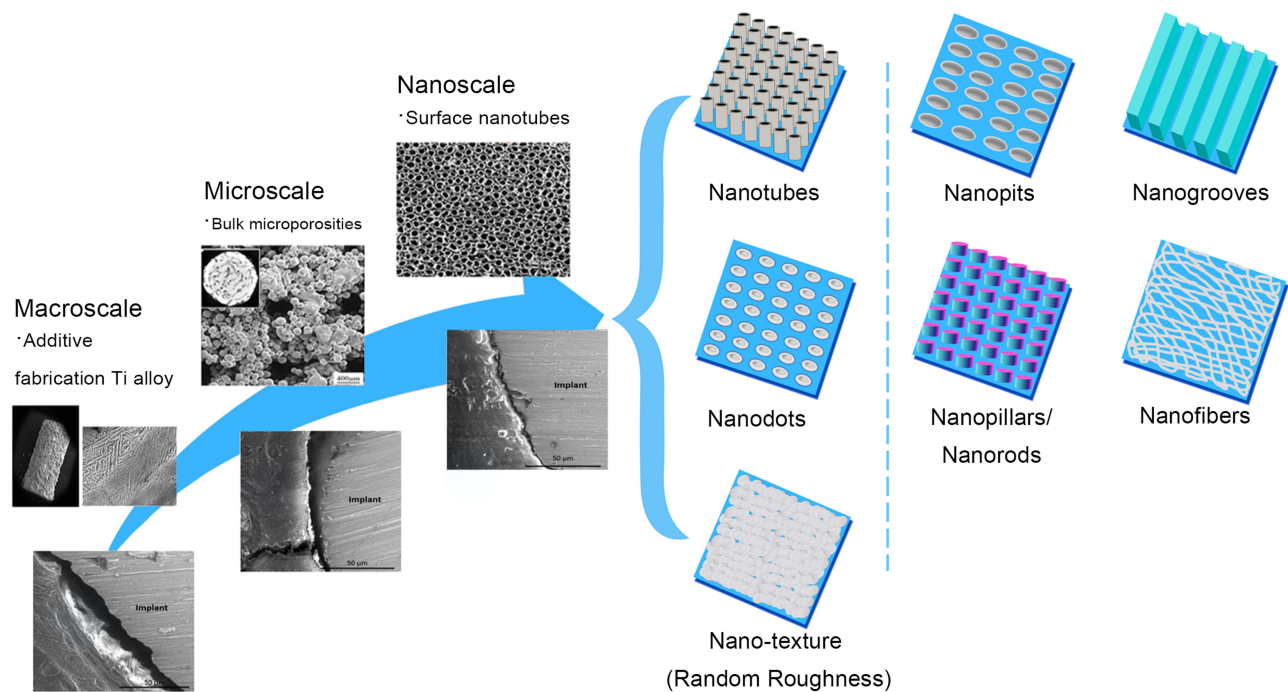
Figure 6 Nanotubes, nano-texture (random roughness), nanodots constructed on nanoscale surface of 3D-printed Ti implants promote osseointegration. Reprinted from *Mater Today*, Volume 45, Mitra I, Bose S, Dernell WS, et al 3D Printing in alloy design to improve biocompatibility in metallic implants. 20–34, Copyright 2021, with permission from Elsevier.[45] *Ann Biomed Eng*, Vol 45(1), Bandyopadhyay A, Shivaram A, Tarafder et al, In Vivo Response of Laser Processed Porous Titanium Implants for Load-Bearing Implants, 249–260, Copyright © 2017, reprinted with permission from SNCSC.[36]

if this observation is of clinical relevance.[83] Furthermore, small animal models like rats and rabbits were used to study the osseointegration of nanostructured 3D-printed Ti implants in all of the selected studies, which possess significant differences in bone structure and remodeling compared to humans. Although the rabbit femur models better simulate the clinical placement of orthopedic implants through both cortical and trabecular bone than the cranial bone onlay models in rats, it is important to validate efficacy with higher-order animal models before transitioning to clinical trials to confirm the optimal surface design and long-term function. The methods of analysis were similar among the included studies and could contribute to the evaluation of osseointegration, but the shape and position of 3D-printed Ti implants were non-negligible variables that affected the osseointegration.

Recently, the clinical implant research community has gained an interest in 3D printing technology and claimed it as a "game changer" in the field, which is a viable method for producing orthopedic implants leading to enhanced biological response, even when compared to a traditionally manufactured, currently used commercial implant.[84,85] In the selected study, laser sintering was used with surface treatments to produce novel Ti-6Al-4V implant surfaces and implants with hierarchical micro- and nano-roughness and hydrophilicity that increased osteoblast response in vitro and demonstrated a significant improvement in cortical bone-implant in rabbit models, when compared to a traditional computer numerical control (CNC).[43] In the last five years, the composite treatment of the nanoscale surface of 3D-printed Ti implants by other physical, chemical, and biological modification methods played a synergistic role in enhancing osseointegration and could be a potential application in the orthopedic field.[37,42,45,86–88] Cohen et al[42] found that the use of the demineralized bone matrix putty (DBX) had an osteoinductive effect, enhancing osseointegration of the roughened surface in the rat calvaria. Bose et al[46] found that 3D-printed porous Ti implants with TiO_2 nanotubes induced early-stage osteogenesis, while the addition of calcium phosphate coating further enhanced defect healing and mechanical interlocking at the interface. However, Cheng et al[47] reported that adding DBX did not significantly enhance mechanical pull-out testing force or total vertical bone volume growth into 3D-printed porous Ti implants, which contradicted those related to the osseointegration effects in other studies. Thus, longer-term and larger animal studies should be performed to confirm the

synergistic effects on osseointegration achieved by composite treatment of the nanoscale surface with other modification methods.

Osseointegration is a dynamic and complex biological process that achieves stable anchorage of an implant by direct bone-to-implant contact, including osteoconduction, de novo bone formation and slow process of bone remodeling.[89,90] Reactions between the surfaces of Ti materials and living tissues are the initial events that occur when Ti implants are placed in vivo. When Ti implants contact the blood of the surgical site, calcium and phosphorus are incorporated into the oxide layer, which promote osseointegration.[91] 3D-printed porous Ti implants are deliberately designed and provide room for new blood vessels and facilitate interlocking between host bone and implants because of their suitable mechanical properties and abundant interconnected macroporous structure. Moreover, the interconnected macroporous structure benefits body fluid circulation, nutrients, oxygen transport, vascularization, and the growth factor accumulation and signaling between osteoblasts and mesenchymal stem cells near the implant site induce bone ingrowth into the implant pores.[16,70] Physicochemical methods have been developed to produce surface roughness and chemical modification of Ti surfaces to increase the adsorption of blood proteins, ions, and molecules, regulate gene expression, and to stimulate blood platelet activation and osteogenic cell migration.[92–94] The physicochemical property of the Ti surface is the crucial factor affecting cellular behaviors and osseointegration, and physical morphology and surface chemistry are not isolated for the modifying of surface morphology will inevitably involve the change in chemical composition at the same time.[24] For example, the improvement of the hydrophilicity of the nanotube surface is not only due to the "capillary effect" of the tube structures but also related to the hydroxyl groups generated after surface modification.[35] The existence of nanotubes has been proven to change the location and spacing of transmembrane integrins, thus affecting the cytoskeleton tension in the actin filaments of the adhered cells.[95] Cell surface integrins, which act as the physical scaffolds between the cytoskeleton and extracellular matrix and sense stimulation of the external environment and regulate cells, have been proven to mediate cell response to biomaterials.[96,97] Studies have shown that integrin α2β1 plays a critical role in osteoblast and mesenchymal response to microscale and nanoscale surface structure and surface energy of Ti substrates.[98,99] Surface modification at the nanoscale surface further enhances biocompatibility of the 3D-printed Ti porous implant surface, thereby promoting better osteoconductive properties, and has been one of the most commonly used methods to promote early-stage osseointegration.[100,101] It helps increase the surface roughness and results in a higher surface area making the contact angle of the surface significantly lower and the surface more hydrophilic, creating a perfect environment for osteoblast cell attachment, maturation, differentiation, and local factor production.[36,102–104] As a result of surface modification, the apatite formation on the surface improves, making it more reactive and osteoconductive, while the nanotopography also promotes osteocyte dendrite formation and maturation which further enhances the 3D formation of osteocyte networks.[105] Furthermore, improved cell adhesion leads to better cell growth and stimulates its differentiation at both protein and gene levels, resulting in strong bonding between the surface and the tissue.[106,107] Studies have reported an up-regulation of pro-osteogenic cell signaling pathways on nanostructured surface including TGF-β/BMP, PGE2, Wnt and Notch, and increased differentiation markers such as alkaline phosphatase (ALP), bone sialoprotein (BSP), and osteocalcin when the hydrophily of nanostructured Ti surfaces improving.[108,109]

This systematic review study has limitations. Although a systematic literature search was performed and the registration of this systematic review was completed before the data extraction was finished, the limited number of studies and heterogeneity in reporting and experimental designs may influence outcomes and hinder result comparison. Due to the above reasons, the optimal surface nanomorphology for promoting the osteointegration of 3D-printed metal implants in vivo cannot be determined.

Conclusion

According to current preclinical studies, irregular nano-texture, nanodots and nanotubes with a diameter of 40–105nm constructed on the surface of porous/solid 3D-printed Ti implants result in better osseointegration and vertical bone ingrowth compared to the untreated/polished ones by significantly promoting cell adhesion, matrix mineralization, and osteogenic differentiation through increased integrin expression. However, the limited number and heterogeneity of current studies influence the determination of the optimal surface nanomorphology of 3D-printed Ti implants. Although

the application of surface morphology modification, coating, and drug or cell loading has brought new opportunities to orthopedic implant design and application, future work need to be focus on the synergistic effects of composite treatment of the nanoscale surface with other modification methods, evaluation of mechanical properties, fatigue and anti-corrosion properties, the durability of the fabricated nanostructures, and consideration of the different roles of physical morphology and chemistry. More standardized and thorough reporting of the nanoscale surface features is also needed to improve our understanding of the surface factors influencing osseointegration in 3D-printed Ti implant applications.

Abbreviations

Ti, Titanium; 3D, Three dimensional; CT, Computed tomography; SLM, Selective laser melting; SLS, Selective laser sintering; EBM, Electron beam melting; LENS, Laser engineered net shaping; DMLS, Direct metal laser sintering; cp Ti, Commercially pure titanium; Ti-6Al-4V, Titanium hexaaluminum tetravanadium; GPa, Gigapascal; ECM, Extracellular matrix; BV, Bone volume; BV/TV, Bone volume fraction; Tb.Th, Trabecular thickness; Tb.N, Trabecular number; Tb.Sp, Trabecular spacing; BIC, Bone to implant contact; SEM, Scanning electron microscopy; LENS™, Laser engineered net shaping; SLS, Selective laser sintering; LSE, Laser surface engineering; AIP, arc ion plating; MAO, Micro-arc oxidation; TiO_2, Titanium dioxide; MSCs, Mesenchymal stem cells; BMP-2, Bone morphogenetic protein 2; BMP-4, Bone morphogenetic protein 4; RUNX2, Runt-related transcription factor 2; VEGF, Vascular endothelial growth factor; CNC, Computer numerical control; DBX, Demineralized bone matrix putty; Ta, Tantalum; OS/BS, Osteoid surface/bone surface.

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Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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