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Literature Review

The effect of alkaline heat treatment on titanium

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ABSTRACT

Background: In recent years, advancements in implant surface modification have garnered considerable interest within the field of biomedical engineering, particularly in dental and orthopaedic implants. High-rise surface modifications demonstrate significant promise in enhancing osseointegration, improving cellular adhesion, and accelerating the healing process. One notable technique, alkaline-heat treatment (AHT), has shown potential for enhancing both the mechanical and biological performance of titanium implants. **Purpose:** The objective of this review is to provide a comprehensive overview of the properties and outcomes associated with alkaline-heat treatment for titanium implants, with a specific focus on the potential benefits for dental applications. **Review:** Based on an analysis of 13 review articles, titanium implants treated with alkaline heat exhibit distinctive properties that enhance their biological efficacy. These include superior osseointegration, improved immunological responses, and heightened antibacterial potential. **Conclusion:** Alkaline-heat treatment significantly enhances titanium implants by creating a nano topography that fosters osseointegration, bolsters immune responses, and exhibits antibacterial effects. These characteristics position AHT as a promising solution for preventing peri-implantitis and facilitating implant healing.

Keywords: Biomaterial; Dental Implant; Titanium; Surface Treatment; Alkaline-Heat Treatment

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INTRODUCTION

Titanium (Ti) is utilized in dental implants due to its capacity to enhance biocompatibility, mechanical properties, and corrosion resistance.[1] Despite these advantages, there remains a 3.11% incidence of implant failure. This failure can occur due to reasons such as excessive surgical site preparation, compromised bone density, thermal damage during drilling, and the absence of antibacterial properties.[2] The absence of antibacterial properties can cause a rise in bacterial infections near the dental implant, leading to inflammation and increased economic burden for the patient.[3] Surface modification is a promising method to enhance the performance of dental implant titanium.[4]

Titanium surface modification involves altering the surface structure and composition to enhance osseointegration, antibacterial properties, biocompatibility, corrosion resistance, and wear.[5] The three primary methods for surface modification are physical, mechanical, and chemical. Chemical interactions are commonly used to create bonds between different functional groups. Compared to physical techniques, chemical methods offer greater benefits due to the covalent bonding of grafted chains to the substrate, which prevents desorption and ensures long-term stability. This approach also enables uniform penetration of chemicals into porous metal structures. Additionally, these treatments transform titanium surfaces, making them bioactive rather than bioinert.[6] Chemical methods involve treating the titanium surface with various chemical reactions, including sol-gel, anodization, chemical vapor deposition, biochemical treatment, and chemical etching treatment.[5,7,8] These methods result in creating micro and nano topography on the titanium surface.

Nanotechnology in dental implants presents groundbreaking advancements, significantly improving the performance and longevity of these medical devices.[9] By engineering surfaces at the nanoscale, it is possible to enhance osseointegration, increase biocompatibility, and reduce the risk of infection.[10] Nanotechnology allows for the precise modification of implant surfaces, fostering better interactions with biological tissues and promoting faster healing processes.[10] Additionally, nanostructured coatings can provide antimicrobial properties, further safeguarding the implant site.[11] Overall, the application of nanotechnology in dental implants represents a transformative approach, offering promising improvements in patient outcomes and implant success rates.[12]

Alkaline-heat treatment (AHT) is a process for modifying the surface of titanium with alkaline etching, followed by heating in a furnace.[5,13] The alkaline solution commonly used for this method is sodium hydroxide (NaOH) or potassium hydroxide (KOH). This method is not only easy to produce and has a lower production cost compared to other techniques, but it also offers numerous biological benefits that significantly enhance dental implant performance.[13] By generating a nanostructured surface that resembles natural cementum, AHT can promote periodontal tissue regeneration similar to that of natural teeth by stimulating the differentiation of periodontal ligament cells into cementoblasts in the area between the implant and the tissue.[13,14] Furthermore, nano topography influences cell behaviors, including proliferation, migration, adhesion, and differentiation, and enhances osseointegration.[15,16] This surface is estimated to ensure implant stability, load-bearing capacity, and overall success in dental implant therapy.[17,18] AHT also demonstrate bactericidal properties.[1] The attributed antibacterial effects are derived from creating a bioactive surface capable of interacting with the surrounding biological environment, thereby bolstering the implant's resistance to bacterial colonization and biofilm formation.[19]

AHT treatments offer numerous advantages in various aspects of dental implants, particularly in enhancing the bioactivity and osseointegration of titanium surfaces.[20] The previous study mainly focused on certain potentials of AHT-treated titanium. However, a comprehensive evaluation is necessary, as the implant will eventually interact with the host cells. The aim of this review is to provide a concise overview of the impact of AHT-modified titanium surfaces, highlighting improvements in surface characteristics, osseointegration, immunology response, and antibacterial potential.[15,16,20–28] Through a detailed examination of current research, the review will illustrate the potential of AHT treatments to advance the field of dental implantology significantly.

REVIEW

The Preferred Reporting Items for Systematic Review and Meta-analysis (PRISMA) were adopted in the review from credible databases, including Science Direct, Google Scholar, and PubMed. The keywords employed were using Boolean operators: (alkali heat treatment) OR (alkaline thermal treatment)) AND (titanium)) AND (implant)). The inclusion criteria for this study encompassed: (1) English-language experimental or research studies focusing on the effects of alkaline-heat treatment on titanium; (2) studies involving the use of NaOH or KOH solutions and a high-temperature furnace in their alkaline-heat method; (3) studies utilizing titanium samples in forms other than powder. Excluded from this review were studies that: (1) were not written in English; (2) were published before January 2019; (3) were duplicate studies; (4) used titanium samples in powder form; (5) did not involve a furnace at the end of the process; (6) employed pre-treatment prior to alkaline-heat; and (7) studies other than the original paper; and (8) irrelevant study. A 5-year review period provides ample time to integrate new findings, technologies, or improvements that could affect the material's evaluation. Additionally, it allows for assessment under various scenarios and adapts to the rapid advancements in materials science. An irrelevant study refers to research that employs the alkaline heat method for purposes other than surface modification of titanium.

423 studies have been identified by authors, with 410 studies ineligible (Figure 1). Data collection was carried out by all the authors. Once the data were gathered, three reviewers were chosen to determine whether to include or exclude the manuscript based on predefined inclusion and exclusion criteria. In cases of disagreement, all reviewers would re-read the manuscript and vote on the final decision. Reviewers assessed the full text rather than just the abstract to mitigate bias. The final decision was based on the majority vote or agreement among the reviewers. The details of data extraction were recorded, and the results were presented in tables exposing the influence of AHT on osseointegration (Table 1), immunological response (Table 2), and antibacterial potency (Table 3).

DISCUSSION

AHT method employed to modify the surface of titanium involves an initial alkaline etching process followed by heating in a furnace.[16,20] This treatment alters titanium's characteristics, significantly impacting its osseointegration (Table 1), immunology response (Table 2), and antibacterial potential (Table 3).[1,15,16,20–28] This review will explain the effects of AHT on these four aspects, providing a comprehensive understanding of its benefits and applications in biomedical implants.

Topography. Most AHT methods result in nano-scale topography on the surface of titanium, which can be observed through scanning electron microscope (SEM).[16] Previous studies have demonstrated that titanium modified by AHT produces multiple nanospikes with a sponge-like inner network structure,[16,20,22] nano-scale petal-like porous network structures,[1] nanoporous,[24] and nanonetworks (Figure 2).[27] Nano topography on titanium surfaces has enhanced various biological processes, improving osseointegration and bone-implant integration.[20] As a result, nano topography offers new possibilities for surface implant modification and holds promise for further enhancing implant performance.[29] It is important to note that AHT may also create other topographies, AHT with heat treatments at different temperatures can result in microstructure,[23] needle-shaped structure,[21] sponge network structure,[15] uniform 3D porous network,[26] uniform porous reticular nanostructure,[28] nanospikes with nanoholes,[22] or distinct nanometric needle-like structures.[23]

Surface Roughness. The application of AHT on titanium surfaces can induce varied surface roughness. Surface roughness parameters (Sp) such as Sa and Ra are

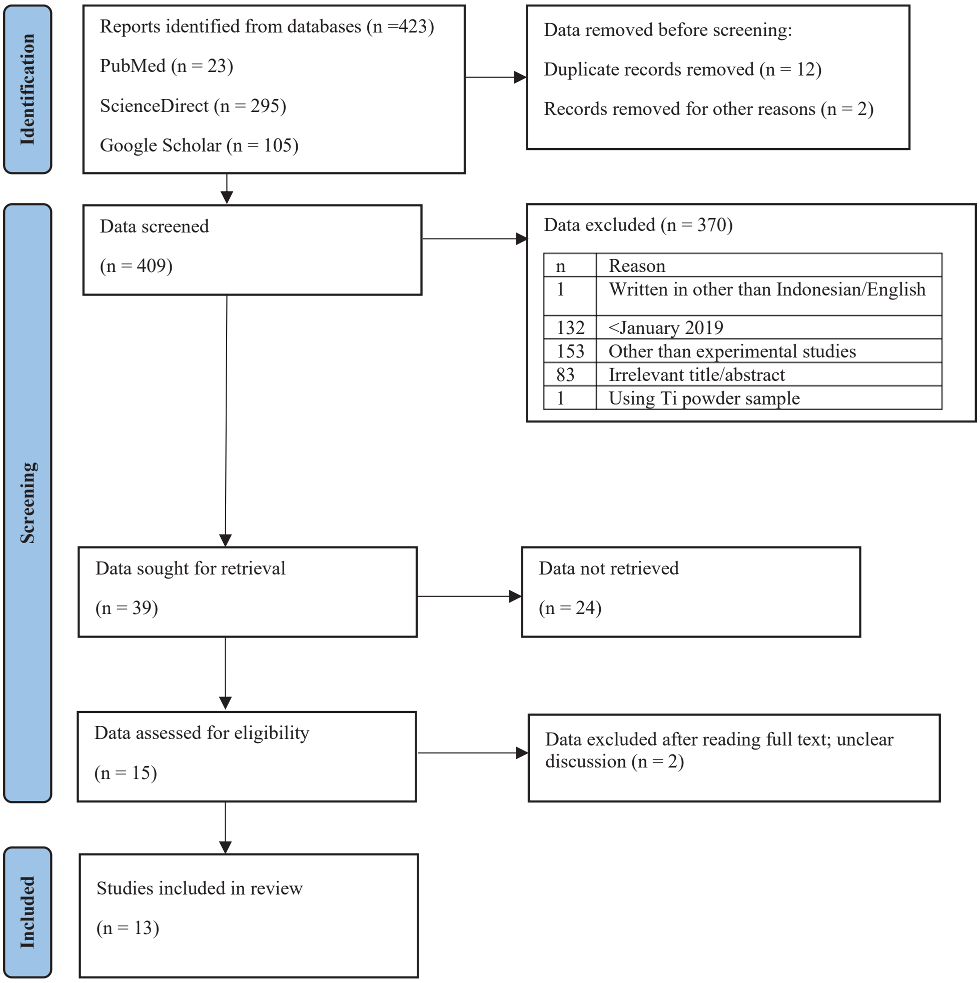

Figure 1. PRISMA's flow chart for this review.

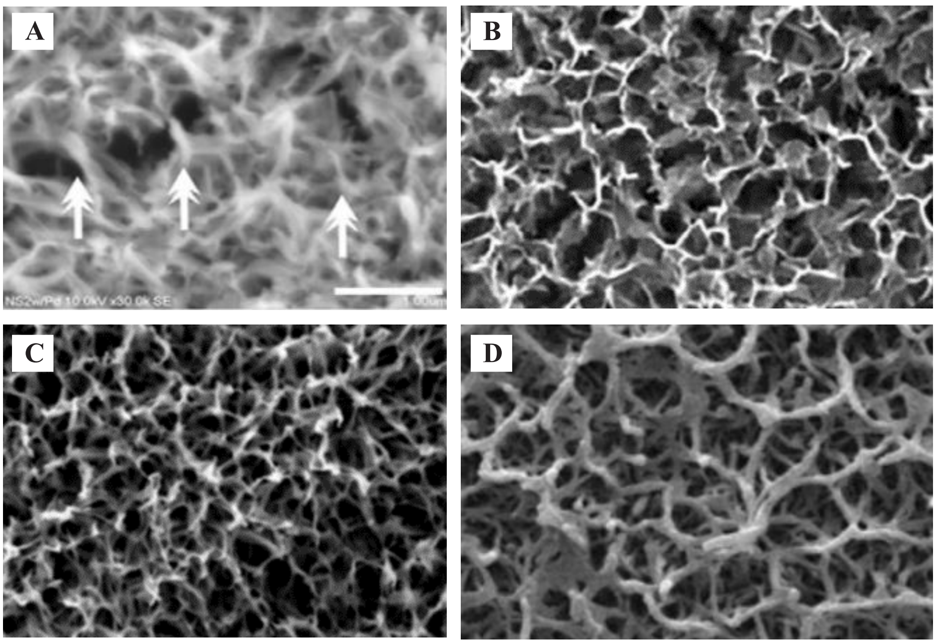

Figure 2. Nanotopography observed through SEM. (A) multiple nanospikes with a sponge-like inner network structure,[20] (B) nano-scale petal-like porous network structures,[1] (C) nanoporous,[24] and (D) nanonetworks.[27] The white arrow indicate the multiple nanospikes.

Table 1. Effect of Alkaline-Heat Treatment in Osseointegration

Ti type	Topography	Surface Roughness	Wetta-bility	Chemical Component	Study Design	Subjects	Results	References
CpTi grade 1	Multiple nano-spikes with sponge like inner network	Sp and Sa higher in AHT treated compare to other group	Super-hydro-philicity	Hydroxyl group	In Vitro	J774A.1 and RAW264.7	AHT induce macrophage into M1 polarization and in inhibit osteoclast precursor become mature osteoclast	Kartikasari et al., 2022
CpTi grade 2	Multiple nano-spikes and crevasses connecting holes with a sponge-like inner network	The AHT titanium surfaces Sa and Sp values were twice as high as the machine surfaces	Super-hydro-philicity	Hydroxyl group	In Vitro	MLO-Y4	Osteocyte maturation is promoted through the production of neuron-like elongated dendrites. In addition, AHT activates paxillin expression, cell-to-cell interactions, and gap junction channels.	He et al., 2022
					In Vivo	11 weeks-old male Sprague-Dawley rats	Following 4 weeks, osteocytes were discovered to be directly connected to the implant surface. Osteocytes created many dendrites with well-developed lacunar-canalicular networks.	
Titanium foils (purity: 99%)	Sponge network structure	Ra value of AHT Ti was significantly higher than Ti	Super-hydro-philicity		In Vitro	MC3T3-E1 and RAW264.7	Nanostructure from AHT facilitates intimate intercellular interaction, provide an advantageous environment for osteoblast growth, and influence the cell morphology	Wang et al., 2020
					In Vivo	80 Sprague Dawley female rats	High bone density and new bone growth were seen when AHT and strontium titanate ($SrTiO_3$) deposition were coupled.	
CpTi grade 4	Microstructure after heat treatment at 600 °C (AHT600) and nanometric needle-like structure after heat treatment 900° C (AHT900)	Surface roughness was higher in AHT 900°C group	Hydro-philic	Sodium titanate (hydroxyl group), rutile	In Vitro		Due to the production of sodium titanate and sodium hydrogen titanate, which enhanced wettability, roughness, and surface area, AHT600 and AHT900 effectively accelerated osseointegration. Additionaly, the AHT triggered the production of apatite.	Oliveira et al., 2021
CpTi	A homogeneous 3D porous network structure, roughly measuring 300-350 nm in size			Amorphous sodium titanate	In vitro		Under conditions of high humidity, the ability of the Ti surface treated with AHT to generate apatite is unstable.	Zhao et al., 2020

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Pure Ti disks	A uniform reticular nano-structure		Hydrophilic			MC3T3-E1	AHT in conjunction with nHA greatly improved cell adhesion and proliferation while also promoting the osteogenic differentiation of MC3T3-E1 cells through the Alkaline Phosphatase (ALP) production.	Wang et al., 2021
CpTi and Ti-5Cu	Nanoscale petal-like porous network structure		Hydrophilic	Anatase and rutile crystal	In Vitro	MC3T3-E1	AHT exhibited noncytotoxic effects, increased initial adhesion, and enhanced osteogenic differentiation of MC3T3-E1 cells.	Zhang et al., 2022
CpTi Grade 2	Nanoporous	Ra was not significantly different between untreated, AHT, and AHT-UV titanium	Super-hydrophilic	Anatase and rutile crystal	In Vitro	HUVECs and RBM cells	AHT improved Human Umbilical Vein Endothelial Cells (HUVECs) and rat bone marrow (RBM) cell adhesion as well as the angiogenic and osteogenic induction capacities	Hatoko et al., 2019
					In Vivo	SD Rats	AHT promoted new bone formation	
Ti machined discs and cylindrical mini-implants, CpTi Grade 5	Nanospikes with nanoholes	Surface roughness was similar between microroughened and AHT surface		Hydroxyl group	In Vivo	Eight-week-old male Wistar rats and 13-weeks-old male Sprague-Dawley rats	Titanium surface emulation Endogenous Periodontal Ligament Cells (PDLCs) are stimulated to differentiate into cementoblasts	Yamada et al., 2022

Table 2. Effect of Alkaline-Heat Treatment in Immunology Response

Ti type	**Topography**	**Surface Roughness**	**Wettability**	**Chemical Component**	**Immunology Response**	**References**
CpTi grade 1	Multiple nano-spikes with sponge like inner network	Sp and Sa higher in AHT treated compare to other group	Superhydrophilicity	Hydroxyl group	AHT induce macrophage into M1 polarization and in inhibit osteoclast precursor become mature osteoclast.	Kartikasari et al., 2022
Commercially pure grade I	Multiple nano-spikes and a sponge-like inner network on the superficial and underlying layers	AHT-treated group had increased Sp and Sa compared to other groups.	Superhydrophilicity	Hydroxyl group	AHT treatment accelerates the synthesis of phagocytosis-related receptors (MARCO, TLR4, TLR2, and SR-A) and increases phagocytosis activity.	Kartikasari et al., 2022

employed to quantify the resulting roughness.[30] The Ra parameter, a two-dimensional (2D) metric, denotes the arithmetic mean of the absolute profile height deviations from the mean line, calculated over a specified assessment length.[30] Conversely, Sa serves as the areal (3D) equivalent of Ra, representing the average height of all measured points within the designated area.[31] The primary distinction lies in Ra's calculation of deviations in one direction after normalizing mean heights for each trace, while Sa calculates deviations from the mean plane across the entire area.[32]

Titanium surfaces modified by AHT yield Sa and Ra values approximately twice as high as those observed on unmodified titanium surfaces.[15,16,20,22,23] Nanonetwork topography on titanium surface has higher surface roughness than nanospike topography.[27] However, the other study explained that Ra was not significantly different

Table 3. Effect of Alkaline-Heat Treatment in Antibacterial Potential

Ti type	Topography	Surface Roughness	Wettability	Chemical Component	Bacteria	Results	References
Plates of CpTi	A needle-shaped structure was formed			Sodium titanate (Na2-Ti5O11) is formed on the Ti surface.	*E. coli* and *S. aureus*	Antibacterial property against *E. coli* and *S. aureus* is improved in combination sample (AHT + bismuth-doped nanohydroxyapatite coatings).	Ciobanu & Harja, 2019
CpTi grade 1 (Ti disc)	Nanospike (1 h alkali-etching) and nanonetwork (16 h alkali-etching)	26.7 Nanospike and 43.7 Nanonetwork	Superhydrophilic		*E. coli, P. aeruginosa*, and *S. aureus*	The antibacterial efficiency of nanonetwork and nanospike varied depending on the strain of bacteria and the geometry of the substrate. Gram-negative bacteria are more vulnerable to nanonetwork, whereas S. aureus is more vulnerable to nanospike.	Ishak et al., 2024
Pure Ti disks	A uniform porous reticular nanostructure		Hydrophilic	Hydroxyl group and amino group	*E. coli* and *S. aureus*	Combination sample (AHT + nHA + nZnO) had a significant inhibitory effect on bacterial growth, 91.06% of *E. coli* and 88.12% of *S. aureus* were inhibited.	Wang et al., 2021
CpTi & Ti-5Cu	Nanoscale petal-like porous network structure was presented on cp-Ti-AH		Hydrophilic	Anatase and rutile crystal	*S. aureus*	AHT combined with Ti-5Cu presented a much stronger antibacterial rate (≥99.99%) than Ti-5Cu without AHT.	Zhang et al., 2022
CpTi Grade 2	Nanoporous	Ra was not significantly different between untreated, AHT, and AHT-UV titanium	Superhydrophilc	Anatase and rutile crystal	*S. aureus*	The biocompatibility and antibacterial qualities of Ti-based materials are enhanced by the combination of heating and UV light. By promoting the generation of ROS, the oral pathogen S. aureus was unable to survive as long on the material surface.	Hatoko et al., 2019

between AHT and unmodified titanium surfaces.[24] Notably, surface roughness is a critical factor in dental implants, as it can bolster mechanical interlocking between the bone and implant, thus enhancing stability and reducing the risk of implant failure.[28,33] In addition, rough surfaces can promote superior cell attachment and proliferation, both of which are essential for establishing a stable bone-implant interface.[33,34]

Wettability. The surface properties of titanium, following modification by AHT, result in a reduced contact angle, specifically less than 10°, in comparison to unmodified titanium surfaces.[27] This static contact angle (θ) serves as an indicator of surface wettability, categorizing surfaces as hydrophobic if θ exceeds 90°, hydrophilic if it is below 90°, and superhydrophilic if it is less than 10°.[35] Multiple studies have shown that AHT-modified titanium surfaces predominantly exhibit superhydrophilic characteristics or possess contact angles below 10° compared to alternative modification methods such as alkali treatment or untreated surfaces.[15,16,20,22] On the contrary, another study also showed that AHT-modified titanium surfaces exhibit hydrophilic characteristics.[1,23,28] Superhydrophilic surfaces attract proteins and osteoblasts, facilitating osteoblast attachment and improving bone integration, which is advantageous for implant osseointegration.[36]

Chemical Component. The modification of AHT-titanium surfaces has been observed to yield chemical components and crystals, hydroxyl groups.[20–22] Hydroxyl groups exhibit a strong correlation with wettability. Surfaces displaying superhydrophilic properties tend to encompass hydroxyl groups, facilitating protein adsorption and cell attachment.[20] Previous result indicates that the alkali treatment process leads to the dissolution of a portion of the TiO_2 layer on the titanium surface by the hydroxyl groups, resulting in the formation of a negatively charged hydrate.[21] This hydrate subsequently interacts with Na^+ ions, giving rise to a sodium titanate hydrogel layer that transforms into sodium titanate crystals after undergoing heat treatment.[21] Furthermore, the heat treatment applied during the AHT process engenders the formation of anatase and rutile crystals, known to function as photocatalysts, effectively hindering bacterial growth.[23,24]

Osseointegration. The direct structural and functional bond between the implant surface and the surrounding bone is known as osseointegration, and it is an important concept in dental implantology. Because it guarantees the durability of the implant within the bone, this procedure is essential for dental implants. In the absence of proper integration, the implant may be at risk of failure due to mobility or insufficient support. Furthermore, osseointegration helps minimize the likelihood of adverse reactions or rejection by the body. Dental implants with successful osseointegration exhibit superior long-term survival rates, often lasting for decades and providing a durable solution for tooth replacement.[17,18] Several studies have demonstrated the effectiveness of AHT on osseointegration.

The main cell type in bone tissue, osteocytes, regulates the actions of osteoblasts and osteoclasts in response to mechanical stress.[37] These fully formed osteocytes have stellate cells that resemble neurons. It is defined as a polygonal or round cell body with numerous long dendritic processes.[38] It is possible for osteocytes in the bone-implant interface region to create lacunar-canalicular networks with osteocytes nearby.[39] AHT takes advantage of the nanotopographical cues to promote the growth of osteocyte lacunar-canalicular networks, which may eventually extend into the surrounding supporting bone near the implant interface.[20] This interconnected network contributes to the augmentation of osseointegration strength for titanium implants.[20]

AHT is reported to inhibits the maturation of osteoclast precursors, thus contributing to osseointegration. AHT induces M1 polarization in macrophages, which leads to the elevation of the production of tumor necrosis factor alpha (TNF-α), granulocyte macrophage colony-stimulating factor (GM-CSF), macrophage colony-stimulating factor (M-CSF), and inducible nitric oxide synthase (iNOS). The enhancement of GM-CSF suppresses the growth of osteoclast precursors into mature osteoclasts, which in turn decreases the quantity of osteoclasts and stimulates osteoblasts to improve osseointegration.[16,22]

A strong soft tissue seal at the implant's transmucosal area is required for sufficient osseointegration strength in order to guarantee long-term clinical stability. By acting as a biological barrier to stop oral germs from penetrating, this seal helps to avoid infection of the tissue surrounding the implant. Collagen fibers such as Sharpey's fibers must be incorporated into the connective tissue in order for the sealing process to take place. The attachment of the epithelium and the dentogingival fibers that connect the connective tissue to the root surface are both necessary for the biological seal to form around the periodontal gingival tissue. The soft tissue seal surrounding the peri-implant tissue follows the same principle.[40] However, at this time, it is unknown how collagen fibers such as Sharpey's fibers found in gingival connective tissue might adhere to an implant's surface directly.[41]

The titanium surface modified by AHT has been found to significantly enhance cellular adhesion and collagen synthesis of gingival fibroblasts. Due to the integration of collagen fibers into the surface structure, this alteration also enhances the binding strength of deposited collagen, making it resistant to inflammatory conditions and experimental overloading. Furthermore, a rabbit maxilla model was used to successfully evaluate the integration of gingival connective tissue onto the surface and the alignment of collagen fiber directions to simulate periodontal tissue.[40] Additionally, AHT has successfully developed a sophisticated titanium nanosurface that emulates the nano topography and micro-mechanical properties of the tooth root cementum (TRC) surface. The research reported here demonstrates that at the interface between tissue and material, the TRC-mimetic titanium surface stimulates endogenous Periodontal Ligament Cells (PDLCs) to differentiate into cementoblasts. Through this innovative method, functional periodontium can be restored on dental implants, similar to the results of autologous tooth transplantation. Notably, such innovation

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is pivotal for inducing dentoalveolar fibrous joints and fostering periodontium regeneration.[22]

AHT formed the apatite formation after 30 days of immersion in Simulated Body Fluid (SBF).[23] Apatite formation in dental implant plays important role in promoting bone biocompatibility, osseointegration, and long-term stability.[42,43] Apatite, a bioactive calcium phosphate, increases mineralization capacity, leading to stronger and more stable bone bonds.[42] It induces cell adhesion, actin ring formation, and bone regeneration, making it a valuable component for implant materials.[44] Moreover, AHT increases the molecular expression of genes associated to osteogenesis, which in turn promotes the mineralization of extracellular matrix (ECM) and the production of bone-like apatite, which in turn may control osteoblast activation and enhance bone regeneration.[1]

AHT can be combined with other treatments such as strontium titanate ($SrTiO_3$) deposition,[15] nano-size hydroxyapatite (nHA),[28] and UV treatment.[24] The combination of AHT and $SrTiO_3$ deposition produces a biomimetic surface substrate that provides the most favorable environment for osteoblast growth, important for osteogenic differentiation and cell morphology.[15] Compared to AHT alone, this combination demonstrated noticeably greater bone density and fresh bone development around implant fixation.[15] Alkaline phosphatase (ALP), a crucial biochemical marker for osteogenic differentiation and the creation of new bone, is expressed more when AHT and nHA are combined.[28] The expression of ALP in the combination of AHT with nHA was reported to be significantly higher than just surface treatment with AHT alone.[28] Combining AHT with UV treatment enhances ALP activity after 7 and 14 days. New bone growth surrounding the implants was encouraged by this as well as elevated osteocalcin (OCN) expression and calcium deposition. This approach was found to be more effective than just modification by AHT alone.[24]

Immunology Response. Macrophages are crucial for both innate immunity and wound healing because they are the first immune cells to come into contact with an implant surface.[45] They also function as osteoclasts' progenitors. The ability of macrophages to generate proinflammatory cytokines and mediators and develop into osteoclasts is strongly influenced by their polarization state.[46] Located at opposite ends of a polarization spectrum that is typical of the anti-inflammatory and tissue-forming macrophages (M2 type, alternative activation macrophages), inflammatory macrophages (M1 type, classical activation macrophages), and inactive macrophages (M0 type). M2 macrophages are essential for inflammation resolution through the synthesis of anti-inflammatory agents and growth factors, while M1 macrophages play a role in host defense during inflammatory conditions by acting as microbial-clearing and antigen-presenting cells through the production of proinflammatory cytokines.[16] AHT treatment has been shown to modulate macrophages towards the M1 type, evidenced by the increase of M1 markers (Interleukin-1β [IL-1β], iNOS, and TNF-α) and decrease of M2 markers (Arginase [Arg1] and cluster of differentiation 206 [CD206]). Research has shown that in human progressive peri-implantitis lesions, M1 macrophages are more prevalent than M2 macrophages.[47,48] Interestingly, M1, but not M2, macrophages suppressed nuclear factor of activated T-cells cytoplasmic 1 (NFATc1), hence impeding osteoclastogenesis and periodontal bone resorption in a mouse periodontitis model.[49] Furthermore, titanium with AHT treatment inhibited the maturation of osteoclast precursor cells following their polarization into M1 macrophages.[16]

Phagocytosis is a vital innate immune mechanism carried out by macrophages that removes pathogens from an affected place.[49] Phagocytosis inhibits the spread of infections and the ensuing inflammatory responses in the affected tissues.[49] In addition to eliminating bacteria, macrophage phagocytosis also eliminates dying neutrophils and combats infections as the initial line of defense.[49] Titanium combined with AHT stimulates the macrophage cell body physically and encourages the development of focal adhesion plaques.[50] These contact stimulations cause the development of receptors linked to phagocytosis, including scavenger receptor-A (SR-A/ CD204), Toll-like Receptor-2 (TLR2), TLR4, and macrophage receptors with collagenous structure (MARCO).[22]

Antibacterial Potential. The antibacterial properties of titanium surfaces modified by AHT have been the subject of extensive research in recent years. AHT has been shown to significantly enhance the antibacterial properties of titanium, making it a promising approach for preventing infection in peri-implant areas and promoting a healthy healing environment.[51–53] The antibacterial effects of this treatment are attributed to creating a bioactive surface that can interact with the surrounding biological environment, enhancing the implant's ability to resist bacterial colonization and biofilm formation.[51,53]

AHT creates nanospikes and nanonetworks structure that susceptible with *Pseudomonas aeruginosa* (*P. aeruginosa*), *Escherichia coli* (*E. coli*), and *Staphylococcus aureus* (*S. aureus*).[27] The study explain that Gram positive bacteria (*S. aureus*) was more susceptible to nanospikes structure, while Gram negative bacteria (*E. coli* and *P. aeruginosa*) were more susceptible to nanonetworks structure. This may be due to the difference in nanostructure density between nanospikes and nanonetworks.[27] High-density nano topography had more contact points with bacteria, which caused more damage to cells, possibly by inducing stress response and membrane deformation.[27]

Combination AHT with other treatment also showed the antibacterial properties. Combination AHT with Cu (Ti-5Cu) increase the antibacterial activity higher compare to AHT alone in *S. aureus*.[1] AHT can also be combined with other treatments such as bismuth-doped nanohydroxyapatite coatings,[21] cerium-doped hydroxyapatite coatings,[25] nano-sized hydroxyapatite (nHA) combine with nano-sized ZnO particles (nZnO),[28] and UV treatment.[24] AHT combined with bismuth-doped nanohydroxyapatite coatings will enhance antibacterial properties against *S. aureus* and *E. coli*.[21] Bismuth-doped nanohydroxyapatite releases bismuth ions to inhibit bacterial proliferation which $Ca^{2+} \leftrightarrow Bi^{3+}$ substitution in the hydroxyapatite lattice that could enhances the antibacterial properties.[21] AHT combined cerium-doped hydroxyapatite coating shows higher antibacterial activity of *S. aureus* (73.59%) and *E. coli* (92.61%).[25] Based on literature, Bacterial cells may experience structural damage and eventual death as a result of interactions between cerium ions in the hydroxyapatite structure and their cell membrane.[54] Other studies also discussed about AHT which combined with nHA combine with nZnO. This combination could kill of *E. coli* (91.06%) and of *S. aureus* (88.12%) due to the reactive oxygen species (ROS) in nZnO, which encourage oxidative damage to the structures of bacteria.[28] Titanium surfaces with AHT combined with UV also shows more effective antibacterial effect with an antibacterial effect of 96% after 6 hours. This produces greater outcomes than simply modifying AHT because UV radiation can produce hydroxyl radicals and superoxide anion, which can damage bacterial membranes and cause intracellular compounds to seep out and bacteria cells to die.[24]

The AHT treatment creates a nanotopography that enhances the performance of titanium implants. This treatment increases osseointegration and boosts immunological and antibacterial responses. Although some studies are still at the in vitro level and further research is needed, this surface modification is one of the best options for preventing peri-implantitis promoting a healthy healing environment to support osseointegration.

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