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Influence of Acid and Alkali Surface Modifications on Titanium Implants: Enhancing Osseointegration and Osteoblast Differentiation

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Abstract

Titanium implants are widely used in biomedical applications due to their excellent biocompatibility and mechanical properties. However, achieving optimal osseointegration remains a challenge. Surface modification techniques, such as acid etching and alkali etching, have been shown to improve implant surface properties, including roughness and chemistry, thereby enhancing cellular adhesion and modulating molecular pathways critical for bone formation. This study evaluated titanium implant surfaces modified using acid etching and alkali etching. Surface topographies were characterized using scanning electron microscopy (SEM), revealing distinct morphologies. Acid-etched surfaces exhibited uniformly roughened structures, while alkali-etched surfaces showed smoother textures with pits. Chemical composition analysis, performed using X-ray photoelectron spectroscopy (XPS), indicated significant alterations, including the formation of bioactive layers that enhance implant integration. In vitro experiments demonstrated that acid-etched surfaces significantly promoted osteoblast adhesion and differentiation compared to alkali-etched surfaces. This was supported by the upregulation of osteogenic molecular markers such as Runx2, SP7, and DLX5, which are vital for bone formation. These findings suggest that acid etching enhances the biological performance of titanium implants, facilitating cellular behaviours necessary for successful osseointegration. In conclusion, acid etching and alkali etching are effective methods for improving titanium implant surfaces, with acid-etched surfaces showing superior potential in promoting osteoblast differentiation and adhesion. Further research is needed to investigate the long-term clinical impact of these surface modifications to optimize implant success and durability.

Keywords: *Acid Etching, Alkali Etching, Osseointegration, Osteoblast Differentiation, Surface Modification, Titanium Implants.*

Introduction

For dental implants to achieve long-term success, osseointegration—defined as the direct structural and functional connection between living bone and the implant surface—is essential. This intricate process involves a complex interplay between the implant's surface properties and the surrounding biological environment.[1,2] The surface quality of an implant is determined by its chemical, physical, mechanical, and topographical characteristics, all of which significantly influence the degree and quality of the bone-to-implant interface. Modifications to implant surfaces have shown remarkable

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potential in improving the extent and robustness of osseointegration [2–5].

Among the biological factors critical to osseointegration, specific groups of proteins, such as the Runt-related transcription factor (Runx) family, play a pivotal role. These transcription factors, comprising Runx1 (AML1/CBFA2), Runx2 (CBFA1/AML3), and Runx3 (AML2/CBFA3), regulate genes involved in diverse cellular processes including bone formation, homeostasis, and response to disease. Runx2, in particular, is indispensable for osteoblast differentiation and skeletal development, making it a key player in bone-related implant integration. By binding DNA, these factors either activate or repress genes, directly influencing the cellular mechanisms underlying osseointegration [6, 7].

Surface composition and roughness are among the critical parameters that govern implant-tissue interactions. Advances in material science have enabled the modification of implant surface topographies at various length scales, including the nanoscale, to enhance osseointegration. Modern dental implants are increasingly designed to incorporate surface features that promote better integration with the surrounding bone. Titanium, due to its exceptional biocompatibility, mechanical strength, and corrosion resistance, has become the gold standard for dental and orthopaedic implants. However, optimizing the surface properties of titanium implants to enhance their biological compatibility remains an area of active research [3, 8].

One promising approach to surface modification involves acid and alkali treatments. Acid modification typically uses aggressive acids such as sulfuric or hydrochloric acid to roughen the surface through sandblasting or chemical etching, creating a micro-roughened texture that promotes better bone adhesion. On the other hand, alkali modification employs alkaline solutions, such as sodium hydroxide, to alter surface chemistry and topography. These treatments not only modify the implant's physical structure but also influence its chemical composition, potentially improving the biological response and implant longevity [9-11].

Similarly, in the context of titanium implants, acid etching selectively removes surface layers, exposing the underlying structure and altering the surface topography. These modifications enhance the material's wettability and promote better interaction with biological tissues [4, 7, 12]. Additionally, acid etching can induce chemical changes by reacting with specific components of the material, further tailoring the surface properties to suit clinical needs.

Alkali treatment, while distinct in its mechanism, similarly offers transformative potential for surface modification. By exposing titanium to an alkaline solution, surface chemistry is altered, creating a favourable environment for calcium phosphate deposition—a precursor to bone formation. The synergistic effects of acid and alkali modifications are of particular interest, as they combine topographical and chemical changes to optimize the implant's performance in vivo [13]. The success of this process is based on selecting an appropriate acid type and concentration with reasonable etching time that can accomplish the required alterations but leave the material structurally intact.

Understanding the morphological changes induced by these treatments is crucial for advancing implant design and improving clinical outcomes. Detailed analysis of surface modifications can shed light on their effects on the metallographic structure of titanium, providing insights into their role in enhancing implant fixation and durability. Such knowledge is essential for guiding future research and developing next-generation implant surfaces.

This study aims to elucidate the morphological changes observed in titanium implants following acid and alkali modifications. By characterizing these changes, the research seeks to provide a deeper understanding of how surface treatments influence implant integration and long-term success. These findings will not only advance the field of implantology but also pave the way for innovative approaches to surface engineering, ultimately contributing to better clinical outcomes for patients.

Materials and Methods

Titanium Implants

Commercially available pure titanium implants of specified dimensions were utilized for this study. These implants are routinely used in dental prosthesis applications.

Acid Etching

To prepare the acid etching solution, a mixture consisting of 30% ultrapure water, 20% hydrochloric acid, and 50% sulfuric acid was diluted by volume. The titanium implants were immersed in this solution, which was preheated to 70 °C, for 30 minutes. Following this treatment, the implants were immediately rinsed with ultrapure water and subjected to ultrasonic cleaning in three cycles, each lasting five minutes. The cleaned samples were then dried in an oven at 101 °C and designated as experimental samples for further analysis.

Alkali Etching

The titanium implants were subjected to an alkali treatment by immersing them in a 5 mol/L sodium hydroxide (NaOH) solution (5 mL of NaOH solution per implant) at 60 °C for 24 hours. Post-treatment, the implants were rinsed gently with ultrapure water and dried in a furnace set to 40 °C for 24 hours. This process ensured proper surface modification and preparation for subsequent cell culture studies.

Cell Culture

Osteoblast Cell Line

An appropriate osteoblast cell line (MG-63), was procured from a cell repository. The cells were cultured following the manufacturer's guidelines, ensuring optimal growth conditions.

Seeding of Cells on Titanium Implants

Titanium implants, both acid-etched, alkali-treated, and untreated controls, were sterilized and placed in cell culture plates. Osteoblast cells were seeded onto the implants, following the recommended protocols for culture medium and incubation conditions one batch in normal media and another in osteogenic media.

Gene Expression Analysis

RNA Extraction

Total RNA was extracted from osteoblast cells adhered to acid-etched, alkali-etched, and control titanium implants using a commercially available RNA extraction kit, following the provided protocol.

Reverse Transcription

The extracted RNA was reverse transcribed into complementary DNA (cDNA) using a reverse transcription kit as per the manufacturer's instructions.

Real-Time Polymerase Chain Reaction (PCR)

Real-time PCR was performed to quantify the expression levels of Runx2 and other relevant genes involved in osteoblast differentiation and bone formation. Appropriate primers and a real-time PCR system were employed for this analysis.

Surface Characterization

Scanning Electron Microscopy (SEM)

The surface morphology and topography of the acid-etched and alkali-etched titanium

implants, as well as the control group, were examined using SEM at a suitable acceleration voltage. Images were captured at various magnifications to assess and compare the surface features.

Statistical Analysis

Data obtained from surface characterization and gene expression analyses were statistically analyzed using Student's t-test to determine significant differences between the acid-etched, alkali-etched, and control groups. Statistical significance was set at $p<0.05$.

Results

Surface Morphology of Titanium Implants

The surface morphology of the titanium implants was characterized using Scanning Electron Microscopy (SEM), and the findings are presented in Figure 1. The naïve titanium implant surface (Figure 1A) exhibited a smooth and unaltered structure, representative of the unmodified material. In contrast, the alkali-modified titanium implant (Figure 1B) displayed irregular patterns and pits, suggesting possible challenges in achieving uniform osseointegration. Notably, the acid-etched titanium implant surface (Figure 1C) revealed a consistently roughened texture, indicating improved surface topography that may favour enhanced interaction with bone tissue. These surface modifications highlight the significant differences in microtopography introduced by alkali and acid treatments, which are critical for their biological performance in osseointegration studies.

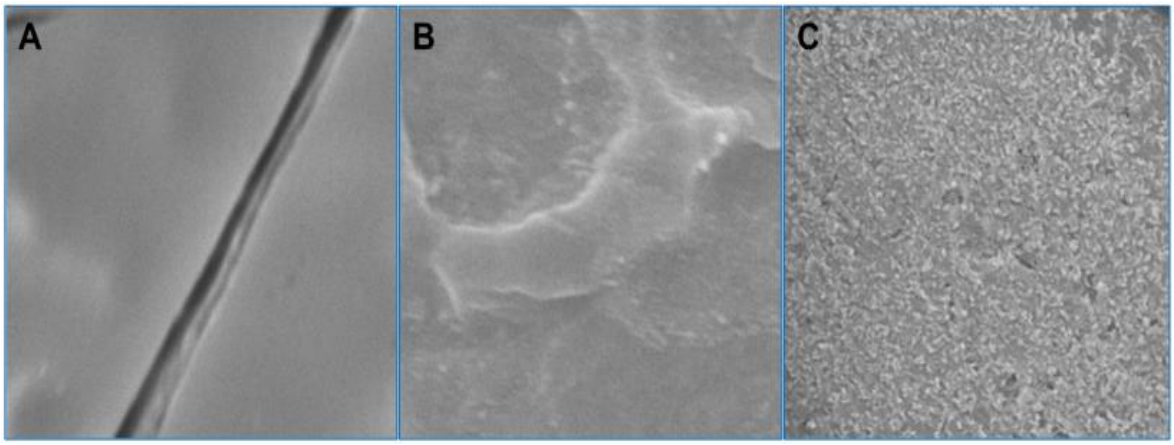

Figure 1. Scanning Electron Microscope (sem) Images of Titanium Implant Surfaces with Different Modifications. (a) Naïve Titanium Implant Surface Showing a Smooth and Unmodified Structure. (b) Titanium Implant with Alkali Surface Modification, Revealing Irregular Patterns and Pits that May Indicate Challenges in Osseointegration. (c) Acid-Etched Titanium Implant Surface Demonstrating a Uniformly Roughened Structure, Potentially Enhancing Osseointegration with Bone

Runx2 Expression in Osteoblasts Cultured on Titanium Implants

The expression of the osteogenic marker Runx2 was evaluated in osteoblasts grown on titanium implants with varying surface modifications under two distinct conditions: normal and osteogenic media (Figure 2). Under normal medium conditions, no significant differences in Runx2 expression were observed among the naïve, alkali-modified, and acid-etched implants. All groups exhibited comparable fold changes, indicating that the baseline osteoblastic activity was unaffected by the surface modifications under non-osteogenic conditions.

However, a significant increase in Runx2 expression was observed in the acid-modified titanium implant group under osteogenic medium conditions (Figure 2). This group showed a marked upregulation of Runx2 expression compared to both the naïve and

alkali-modified implants ($p < 0.05$). The naïve and alkali-modified groups, in contrast, demonstrated no significant changes in expression levels under osteogenic conditions. The enhanced Runx2 expression in the acid-modified group suggests that the uniformly roughened surface generated by acid etching provides a favourable microenvironment for osteoblast differentiation, particularly under osteogenic stimuli.

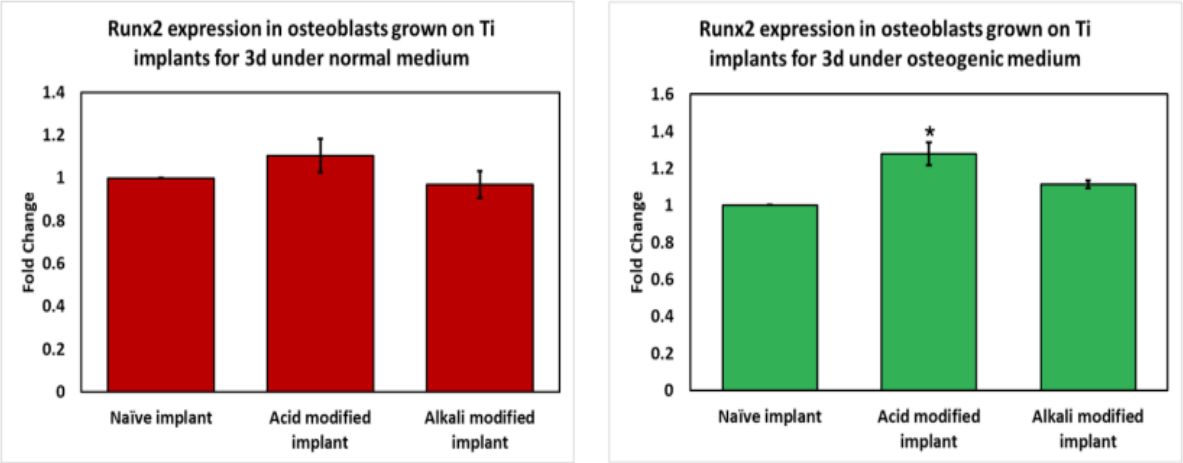

Figure 2. Runx2 Expression in Osteoblasts Grown on Titanium Implants Under Normal and Osteogenic Media for 3 Days. The Bar Graphs Represent the Fold Change in Runx2 Gene Expression in Osteoblasts Seeded on Naïve, Acid-Modified, and Alkali-Modified Titanium Implants. The Left Graph Shows Results Under Normal Culture Conditions, While the Right Graph Corresponds to Osteogenic Conditions. A Significant Increase in Runx2 Expression was Observed in the Acid-Modified Implants Compared to Naïve and Alkali-Modified Implants Under Osteogenic Conditions (*). No Significant Differences were Observed Among the Groups Under Normal Conditions

Comparative Analysis of Surface Modification Effects

These findings collectively underscore the critical role of surface modification in influencing cellular responses to titanium implants. While alkali modification introduced irregular and inconsistent surface features that did not significantly impact Runx2 expression, acid etching created a uniformly roughened surface that enhanced osteoblastic activity and differentiation, as evidenced by the elevated expression of Runx2. This enhanced osteogenic response to acid-etched implants suggests their superior potential for applications where rapid and effective osseointegration is required.

Discussion

An expanding body of research has provided substantial insights into the morphological and chemical alterations induced by surface modification processes and their impact on implant performance. Acid and alkali modifications of titanium implant surfaces have been extensively studied to optimize osseointegration. For example, Baima et al. (2024) demonstrated that acid modification significantly increased surface roughness, which in turn enhanced cell adhesion and accelerated osseointegration. These findings highlight the importance of surface microtopography in fostering a conducive environment for bone-implant interaction [14].

Advanced imaging techniques, including atomic force microscopy (AFM) and scanning electron microscopy (SEM), have been pivotal in uncovering microstructural changes induced by acid and alkali modifications. These studies have revealed critical surface features, such as

increased porosity and irregularities, which are strongly associated with improved osseointegration potential [10]. The SEM analysis in our study revealed that acid-etched titanium implants exhibited a uniformly roughened surface, which is advantageous for cellular adhesion and subsequent bone formation. In contrast, alkali-modified surfaces displayed smoother topography interspersed with pits, which may limit their effectiveness in promoting osseointegration compared to acid-etched surfaces.

Chemical characterization techniques, such as X-ray photoelectron spectroscopy (XPS), have further elucidated the elemental composition and surface chemistry changes resulting from modification processes. For instance, Ochi et al. (2021) reported the development of bioactive layers on modified implant surfaces, which play a crucial role in facilitating the integration of the implant with surrounding bone tissue. These findings underscore the interplay between chemical and physical properties in determining implant success [15].

The molecular mechanisms governing osseointegration have also been linked to the activity of Runx molecules, which are key regulators of osteoblast differentiation and bone formation. Runx2, in particular, is recognized as a master transcription factor that orchestrates the expression of critical genes involved in osteogenesis, including collagen and osteocalcin. The upregulation of Runx2 observed in osteogenic conditions on acid-etched titanium surfaces in our study suggests that the roughened microtopography enhances osteoblast differentiation and bone matrix deposition. This observation aligns with prior research demonstrating that nanostructured surfaces promote osteoblastic activity and improve the bone-implant interface by minimizing the formation of fibrous tissue barriers [15, 16].

Furthermore, studies have highlighted the necessity of precise regulation of Runx2 and its downstream pathways to ensure successful osseointegration. Runx2 activation initiates a cascade of events leading to mineralization and the stabilization of the bone-implant interface. This regulation is essential for achieving the mechanical stability required for long-term implant durability. For example, Li et al. (2007) demonstrated that mesenchymal stem cells differentiate into osteoblasts under the influence of Runx2, further underscoring its critical role in osteoblastogenesis [17].

Our findings align with previous research, such as that of Divya Rani et al., who demonstrated enhanced initial osteoblast adhesion on nanostructured titanium implants formed using hydrothermal processes. Similarly, Liao et al. (2023) reported the expression of osteogenic markers such as SP7, DLX5, and CTNNB1 in human mesenchymal stem cells cultured on modified implant surfaces, further supporting the role of surface modifications in promoting osteogenesis. These studies emphasize the need to integrate chemical and topographical modifications to achieve optimal outcomes [18].

Finally, the mechanical interaction between the implant surface and bone tissue is a crucial determinant of implant integration. A well-modified implant surface fosters direct bone contact, allowing the implant to function as an integral part of the skeletal system. Acid-etched titanium implants, with their uniformly roughened structure, provide an ideal microenvironment for this interaction, as observed in our study. This suggests that acid-etched surfaces can significantly improve the functional activity of osteoblasts at the bone-implant interface, reducing the risk of fibrous tissue formation and enhancing implant stability.

In conclusion, the interplay between implant surface modifications, Runx2 activity, and osteogenic gene expression is central to improving implant success rates. By advancing our understanding of these processes, we can design more effective

implant surfaces that promote rapid and reliable osseointegration, ultimately leading to better clinical outcomes and long-term durability. Future studies should focus on integrating advanced surface characterization techniques and molecular analyses to elucidate these complex interactions further.

Conclusion

Acid etching and alkali etching are effective surface modification techniques for optimizing titanium implant surfaces to enhance osseointegration. Acid-etched surfaces, with their uniformly roughened topography, promote superior cellular adhesion, proliferation, and differentiation, facilitating bone matrix deposition and integration. In contrast, alkali-etched surfaces exhibit smoother topologies with localized pits, contributing to a different cellular response. These modifications influence surface roughness and chemistry and activate key molecular pathways, such as Runx2, which play a critical role in osteoblast differentiation and bone formation. While acid-etched surfaces demonstrate greater potential for osseointegration, further research is required to elucidate the molecular mechanisms and signalling pathways involved fully. Advanced characterization techniques and long-term clinical evaluations will be pivotal in validating the efficacy and safety of these modifications, ultimately guiding the development of more effective and durable implant designs.

Conflict of Interest

Nil.

Acknowledgements

Nil.

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