

Developing Biomimetic Surface Structures at the nanoscale on Metallic Implants for Enhanced Functionality

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INTRODUCTION: Dental implants, commonly used to replace missing teeth, and load-bearing orthopedic implants encounter a significant challenge with localized bacterial accumulation on the implant surface. This bacterial buildup plays a critical role in the developing and progressing biofilms that are difficult to remove and ultimately lead to implant failure. Despite the widespread use of metallic implants, no implant material has entirely prevented bacterial infection and provided bone osseointegration. Ongoing research is focused on exploring surface treatments to enhance bioactivity and osseointegration while concurrently addressing the prevention of bacterial adhesion. In this study, we attempted to biomimetically engineer the nanostructured surface of the titanium (Ti) alloy, inspired by the natural antibacterial nanostructure found in the cicada's wing, using alkaline hydrothermal treatment (AHT).

METHODS: Titanium (Ti) Grade 5 (Ti6Al4V) sheets with dimensions (6" X 6" X 0.016") were cut into small, flat, square sections that were sanded progressively to a mirror-shine polish. Using an alkaline hydrothermal treatment (AHT) at a temperature of 150°C and 5 N, NaOH concentration, and five different time durations (30 minutes, 1-hour, 2-hours, 4-hours, and 8-hours), five distinct surfaces with unique nanopillar structure were developed. Following the alkaline hydrothermal treatment (AHT), the samples underwent annealing in a furnace at 900°C for 2 hours. Subsequently, surface characterization was performed to compare the surface topology of the treated groups using a profilometer, and wettability was assessed by measuring contact angles with a goniometer. Further, to examine the impact of surface treatment on extracellular matrix mineralization, Ti sheets were autoclave-sterilized and seeded with human osteoblast cells (hFOB) at a density of 5×10^4 cells/cm² per well in the growth medium. After 24 hours, the medium was replaced with the osteogenic differentiation medium and refreshed every two days. On day 7, mineralization of the extracellular matrix was assessed through Alizarin red staining. Cells were fixed with 4% paraformaldehyde for 30 minutes and stained with Alizarin red solution for 1 hour at room temperature. Stained samples were thoroughly washed with dH₂O water and quantified by adding 10% cetylpyridinium chloride solution to each sample. After overnight incubation at room temperature, 200 µL of the eluted solution was transferred to a 96-well plate, and absorbance at 562 nm was measured using a microplate reader.

RESULTS SECTION: The control group (Ti group without any treatment) exhibited a relatively smooth surface with a roughness value of 58 ± 1.7 nm, significantly different from the treatment groups. The average roughness values for the 30-minute and 1-hour AHT groups were 423.74 ± 1.4 nm and 537.6 ± 15.3 nm, respectively—both statistically lower than the 8-hour AHT group, with an average roughness of 988.5 ± 59.9 nm. For the control group, the average contact angle measured was $72.56 \pm 5.6^\circ$, and there was a statistically significant decrease in the contact angle for the treated groups compared to the control. The average contact angle measurements for the 30-minute, 1-hour, 2-hours, 4-hours, and 8-hours AHT groups were $24.01 \pm 1.95^\circ$, $16.92 \pm 1.95^\circ$, $14.34 \pm 1.06^\circ$, $9.89 \pm 0.55^\circ$, and $7.00 \pm 0.2^\circ$, respectively. Except for the 1-hour and 2-hour AHT groups, there was a statistically significant difference in contact angles among all groups, indicating successful modification and enhanced hydrophilic properties for improved cell adhesion and attachment. The osteogenic differentiation of hFOB cells in the osteogenic medium was assessed, revealing that the AHT-treated Ti group exhibited a preference for the mineralization of the extracellular matrix compared to the untreated Ti-negative group. This observation was consistent with the results of colorimetric quantitative measurements. Notably, only the 8-hour AHT group showed a statistically significant difference ($P < 0.05$) compared to the negative control. In comparison, the TCPS positive control, where cells were grown on the well surface of a 24-well plate, demonstrated the highest absorbance among the groups.

DISCUSSION: Our initial findings suggest that adjusting concentration, processing time, and temperature during the AHT process enables the creation of diverse biomimetic nanotextures. These nanopillars on the surfaces of Ti alloy have shown to enhance the mineralization of the extracellular matrix, indicating their potential to promote the differentiation of bone cells. We observed a statistically significant increase ($P < 0.05$) in roughness for the treated groups with longer AHT durations. This was attributed to a dissolution-precipitation process involving the TiO₂ layer, followed by the precipitation of TiO₂ nanoparticles on the surface, coalescing to form nanopillars. Surface wettability analysis also demonstrated increased hydrophilicity with longer AHT durations. Given that cell adhesion relies on surface wettability and roughness, influencing cell-cell and cell-surface interactions and cell behavior [1,2], we anticipate that AHT treatment creates a more favorable surface for bone cells to attach and proliferate. While these preliminary results are promising, further investigations are crucial to optimize these parameters for surfaces that not only exhibit antibacterial properties but also support enhanced cell adhesion and proliferation, thereby facilitating improved integration.

SIGNIFICANCE/CLINICAL RELEVANCE: The positive results observed with nanotextured implant coatings on implant materials highlight the importance of examining their behavior and performance in the oral environment. This investigation is crucial for gaining insights into the long-term stability of surface nanostructures and their clinical effectiveness in promoting osteogenesis while preventing bacterial accumulation and infection.

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