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In vitro development and investigation of a novel nano-structured biomaterial implant coating: An original research

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Abstract

Background: Dental implant therapy represents a rapidly evolving and promising area in the restoration of both completely and partially edentulous arches. The surfaces of implants have been established to exert a critical influence on molecular interactions, cellular responses, and Osseo integration. As a result, researchers worldwide have been engaged in developing second-generation implants with surfaces designed to enhance and expedite implant Osseointegration. Moreover, ongoing studies are exploring the potential of coating implant surfaces with biomaterials to inhibit bacterial colonization.

Aim: The aim of this study is to develop biomaterial coating in in-vitro models and to test the efficacy of the coated implant abutment interfaces in providing hermetic seal to microbial flux.

Materials and Methods: In this study, two types of phosphonato-silane coatings were formulated, employing combinations of Glycidoxy-

propyltrimethoxy-silane (GPTMS) and Methyltriethoxy-silane (MTEOS) to create a biocompatible protective coating for implants. This approach draws inspiration from strategies used in the machinery industry, focusing on enhancing the frictional engagement between components to prevent inadvertent separation. The study involved dividing the samples into three groups: the control group without surface treatment, Group 1 without alkali pre-treatment, and Group 2 with alkali pre-treatment. Subsequently, Group 1 and Group 2 underwent a series of processes, including exposure to the above solution, drying at room temperature for 15 minutes, and heat curing for 1 hour at 120°C. The presence of bacteria was then detected using polymerase chain reaction (PCR) analysis.

Results: Based on the XPS analysis, both Group 1 (without alkali pre-treatment) and Group 2 (with alkali pre-treatment) exhibit a substantial atomic concentration of silicon. Notably, Group 1demonstrates a silicon concentration of 12.25 atomic percent, which is twice the 6.56 atomic percent concentration observed in Group 2. No bacterial colonisation was observed in any of the groups indicating hermetic seal to microbial flux.

Conclusion: Considering these findings, there is potential for strategies aimed at coating implant surfaces to prevent initial bacterial attachment, thus potentially decreasing the occurrence of peri- implantitis, particularly in patient groups at higher risk.

Keywords: Polymerase Chain Reaction, GPTMS, MTEOS.

Introduction

Itanium is widely regarded as the gold standard implant material within the field of implantology. It is worth noting that both pure titanium (Ti) and titanium alloys are extensively utilized in this domain. Specifically, there exist four unalloyed grades of commercially pure titanium (CP Ti), each distinguished by the concentration of impurities present [1]. It is interesting to observe that the elastic modulus of CP Ti is comparable to that of tooth enamel and noble alloys, albeit lower than that of other base metals [2].Exceptional corrosion resistance makes CP Ti favoured for applications where high strength is unnecessary [3]. Surface conditioning enhances micro retention, while early clinical titanium implants featured a smooth surface texture. Implant surfaces play a critical role in molecular interactions, cellular response, and osseointegration, leading to the development of second-generation implants aimed at improving osseointegration. Surface modification aims to promote osseointegration, facilitating faster and stronger bone formation for enhanced stability during the healing process, particularly in areas with poor bone quality and quantity. Advances in microbiology and nanotechnology have advanced surface engineering in implant dentistry. Surface roughness influences cell migration and proliferation, affecting bone-to-implant contact (BIC), and suggesting the implant's microstructure influences biomaterial tissue interaction. Additionally, various biomaterials may negatively impact microbial colonization and microleakage through micro-gaps [4,5].Biomedical implants have revolutionized modern medicine, providing solutions for a range of health conditions from orthopedic repairs to dental restorations. The field of biomedical engineering constantly seeks innovative solutions to enhance implant performance and longevity. Nano-structured biomaterial coatings have emerged as a promising avenue, particularly the combination of GPTMS and MTEOS silanes, which has garnered at tension for its potential in revolutionizing implant coatings [6, 7]. This article explores groundbreaking research focusing on the in vitro

development and investigation of a novel nano-structured biomaterial implant coating and its correlation with bacterial colonization.

The study embarked on developing a cutting-edge nano-structured biomaterial implant coating using GPTMS and MTEOS silanes. These silanes, known for their biocompatibility and nano-structuring capabilities, were precisely selected to create a robust coating that could enhance the performance of biomedical implants. Using in vitro models, the coating was meticulously crafted and characterized, with thorough evaluations conducted on its surface morphology, chemical composition, and antibacterial effects. Central to the investigation was the correlation between the novel coating and bacterial colonization, a crucial factor in implant success. By simulating microbial flux in vitro, the efficacy of the coated implant abutment interfaces in providing a hermetic seal against bacterial infiltration was assessed. This aspect sheds light on the coating's potential in mitigating infection risks associated with biomedical implants, a significant concern in clinical settings. Thus the aim of this study is to develop biomaterial coating in in-vitro models and to test the efficacy of the coated implant abutment interfaces in providing hermetic seal to microbial flux.

Methodology

In this study, a comprehensive investigation was conducted utilizing a total of six titanium implants, each precisely measuring 4.2mm in diameter and 8mm in length. These implants were meticulously divided into distinct groups to explore the effects of various surface treatments. The control group consisted of implants devoid of any surface treatment, while Group 1 comprised implants subjected to treatment without prior alkali pre-treatment. Conversely, Group 2 implants underwent a thorough alkali pre-treatment process.

The experimental coatings were prepared by mixing GPTMS and MTEOS solutions in water, maintaining a precise ratio of 3:1 (v/v). For Group 1 implants, the coating procedure involved immersion in the GPTMS and MTEOS solution for a designated period of 20 minutes, followed by subsequent drying at room temperature for 15 minutes, and ultimately heat curing for one hour at a temperature of 120 degrees Celsius.

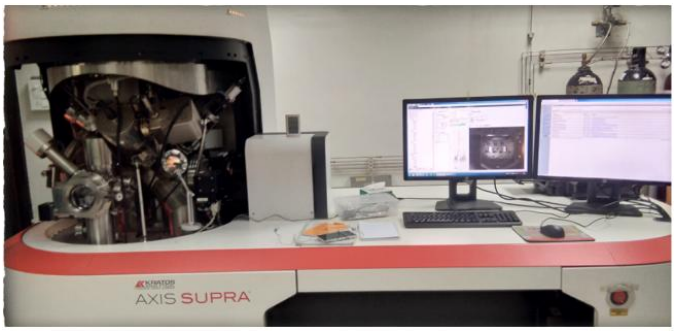

Figure 1: Chemical characterisation of investigated implants

In contrast, Group 2 implants underwent a more elaborate treatment regimen, starting with a 24-hour immersion in 3 Molar NaOH solution, followed by neutralization using 3N acetic acid. Post-neutralization, the implants were dried at room temperature before undergoing immersion in the GPTMS and MTEOS solution for 45 minutes. Subsequently, they were dried again at room temperature for 15 minutes and heat-cured for one hour at 120 degrees Celsius.

Following these treatment procedures, the implants were meticulously analyzed using X-ray photoelectron spectroscopy (XPS) to characterize the chemical composition of the coatings. (Figure 1). Furthermore, the efficacy of these coatings in providing a hermetic seal was thoroughly assessed by employing polymerase chain reaction (PCR) analysis to detect the presence of bacteria.

In the PCR analysis all three dental implants were placed in 10 mL of nutrient broth and incubated at 37°C for 24

hours, resulting in bacterial growth in each broth. Samples from the grown broth were streaked onto separate nutrient agar plates, and examination revealed similar colony morphologies on plates 1 and 2. Consequently, only one isolate from each of these plates was chosen for further analysis.

DNA isolation was then performed on the selected single colony from each plate using the Boiling lysis method, which involves exposing bacterial cells to high temperatures to break their cell walls and release DNA. The PCR analysis utilized the 8F/806R primers designed to amplify specific regions of bacterial DNA. Subsequent to PCR amplification, single-stranded DNA sequencing was conducted with the 8F primer, facilitating the determination of the nucleotide sequence of the amplified DNA fragment and providing crucial insights into the bacterial species present on the dental implants.

Results

The chemical characterization of the investigated implants was conducted using X-ray Photoelectron Spectroscopy (XPS) with the Kratos Analytical Axis Supra instrument, housed at IIT Bombay. This advanced analytical technique, facilitated by the Shimadzu group, allowed for precise analysis of the implants' chemical composition.

The results of the analysis are summarized in Table 1, providing insights into the relative chemical composition, measured in atomic concentration (at. conc.), as determined by XPS analysis.

Group	Ti (at. %)	O (at. %)	C (at. %)	Si (at. %)	Na (at. %)
Control	3.90	29.83	66.27	0.00	0.00
Group 1	0.38	29.90	57.47	12.25	0.00
Group 2	4.56	29.20	59.02	6.56	0.067

Table 1: Relative chemical composition (at. %) determined by XPS analysis of Phosphonato- silane coated alloy samples

BINDING ENERGY	ELEMENT	NAME/CLASS
CONTROL		
284.5 eV	C	Polymer
458.5 eV	Ti	Titanium oxide
531.0 eV	V	Vanadium oxide
GROUP 1		
102.5 eV	O	Di Aluminum trioxide
285.5 eV	C	Polymer
535.5 eV	Si	Silicon dioxide
GROUP 2		
284.5 Ev	C	Polymer
459.6 eV	Ti	Titanium oxide
532.5 eV	Si	Silicon dioxide

Table 2: Chemical compositions and classes of materials present on various groups.

Table 1 and Table 2 unveiled distinct differences in their chemical composition across the control group and Groups 1 and 2. Notably, Group 1 exhibited a significantly lower percentage of titanium compared to both the control and Group 2, indicating a potential reduction in titanium content resulting from the surface treatment in Group 1.

Regarding carbon, Group 1 displayed a lower percentage compared to the control, while Group 2 showed a slight increase, implying a potential reduction of carbon content in Group 1 and a minor increase in Group 2 following surface treatment.

Additionally, the presence of silicon was detected in both Group 1 and Group 2, whereas it was absent in the control group, indicating the introduction of silicon onto the alloy samples through surface treatment, with Group 1 exhibiting a higher percentage compared to Group 2.

Interestingly, the oxygen content remained relatively consistent across all groups, suggesting that the surface treatment had minimal impact on the presence of oxygen.

Furthermore, Group 2 displayed a small percentage of sodium, which was absent in both the control and Group 1, suggesting the introduction of sodium onto the alloy samples specifically in Group 2 through surface treatment.

Table 3 presents the results of PCR analysis regarding bacterial colonization by the red complex species (Porphyromonas gingivalis, Treponema denticola, Tannerella forsythia) across different samples. The samples are labelled as Control, 1, and 2, with each corresponding to a specific sequencing primer (16S). The results are categorized as either "Pass" or "Fail" based on the presence or absence of the red complex species.

In this analysis, all samples, including the Control and those treated with different surface coatings (1 and 2), passed the test, indicating the absence of the red complex species. These findings provide important insights into the efficacy of the surface treatments in mitigating bacterial colonization by the red complex species, which are known for their pathogenic potential in dental settings.

Sample Name	Sequencing primer	Result (Pass/Fail)
Control	16S	Pass
Group 1	16S	Pass
Group 2	16S	Pass

Table 3: PCR analysis results concerning bacterial colonisation by red complex.

Binding energy curve is the one which depicts the stability of the nucleus. It is the energy required to break the bond between two molecules. From the binding energy curves and the data it is evident that in all the groups the polymer has created a strong bond. Also it is having same binding energy in all the groups.

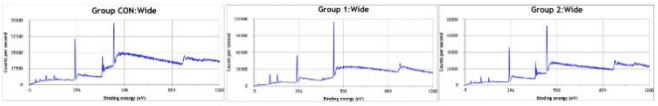

Figure 2: Binding energy of 3 groups

Discussion

In contemporary practice, an array of materials is employed for surface coating over implants, with diverse methodologies available for surface modification of implants. These methodologies encompass techniques such as titanium plasma spray, hydroxyapatite coating, bioactive glass and ceramics, growth factors, and fluoride treatment. The rationale behind surface modification of implants encompasses objectives such as augmenting bioactivity to mitigate peri implantitis, amplifying functional surface area, fostering adhesion and proliferation of osteoblasts, and evincing high surface energy.

Macro surface modifications are intricately linked to implant design and topography, while Micro surface modifications encompass methodologies like sand blasting, acid etching, and other chemical treatments, as well as techniques such as electrophoresis, titanium plasma spray, biomimetic deposition, and laser ablation. It is postulated that the nano topography of dental implants serves to refine cell implant interaction and potentially influences osteoblastic activity, thereby mitigating bacterial ingress and the incidence of peri-implantitis [8]. Peri- implantitis, an inflammatory disease due to bacterial colonization and plaque formation on implant surfaces, precipitates bone resorption and impairs osseointegration [9,10]. Strategies aimed at coating implant surfaces to preclude initial bacterial attachment may play a pivotal role in

mitigating peri-implantitis, particularly among vulnerable patient cohorts. Thus the current investigation endeavours to develop two variants of Phosphonato-silane coatings. This Study exemplifies the utilization of combination of Glycidoxypropyltrimethoxy-silane (GPTMS) with Methyltriethoxy-silane (MTEOS) based Biocompatible protective coatings, which are seamlessly compatible with bodily fluids and potentiate cell proliferation on the treated specimens. This innovative approach draws inspiration from industrial practices, with the aim of enhancing the frictional engagement between components and forestalling inadvertent separation.

In this study, the XPS analysis data from Tables 1, 2, and PCR analysis from Table 3 provide valuable insights into the effectiveness of the novel biomaterial impregnation with no bacterial colonisation on the implants. Firstly, the higher titanium content observed in the Control and Group 2 suggests a denser or less altered titanium surface, indicating minimal surface modifications. Conversely, Group 1, with lower titanium content, may have undergone surface modifications due to the biomaterial impregnation process.

Secondly, the notably higher silicon content observed in Group 1 suggests successful silane deposition, as this group underwent GPTMS and MTEOS phosphonosilane impregnation, potentially resulting in the formation of a silicon-rich surface layer. Conversely, while Group2 also indicates successful silane deposition, the silicon content is found to be lower compared to Group 1. This disparity may be attributed to the alkali treatment undergone by Group 2 prior to the silane coating process, potentially affecting the silane deposition efficacy.

Thirdly, the decreased carbon content in Group 1 and Group 2 suggests changes in surface chemistry due to the biomaterial impregnation process, which may alter surface hydrophobicity / hydrophilicity. Lastly, the minor presence of sodium in Group 2 may suggest interaction with the coating process, possibly due to the alkali treatment in Group 2.

The red complex bacteria are commonly associated with periodontal diseases, particularly severe forms like periodontitis.

These bacteria have been identified as key pathogens in the development and progression of periodontal disease due to their synergistic interactions and ability to evade host immune responses. Therefore in this study we have chosen the red complex bacteria for testing bacterial colonization in PCR analysis providing a focused assessment of potential pathogens known to be involved in periodontal diseases, offering valuable insights into the oral health implications of the studied biomaterials or treatments. Notably, no bacterial colonization was observed in any of the groups, indicating the effectiveness of the biomaterial impregnation in providing hermetic seal to microbial flux.

The study demonstrates several strengths, including its innovative approach through the introduction of two variants of phosphonato-silane coatings, offering a novel method for surface modification of implants. The use of Glycidoxypropyltrimethoxy-silane (GPTMS) and Methyltriethoxy-silane (MTEOS) based coatings showcases compatibility with bodily fluids and fosters cell proliferation on treated specimens, suggesting potential biocompatibility and safety.

However, limitations exist, such as the lack of clinical validation, which restricts the generalization of findings to clinical settings. Additionally, the study primarily focuses on short-term outcomes, overlooking the long-term effects of the novel surface modifications on implant performance and bacterial colonization,

underscoring the necessity for further long- term investigations.

Conclusion

In conclusion, this study explores innovative Phosphonato-silane coatings, combining GPTMS and MTEOS, for implant surface modification. XPS and PCR analyses reveal the effectiveness of these coatings in reducing bacterial colonization on implants. The XPS analysis reveals a notable concentration of silicon in both Group 1 and Group 2, with Group 1 exhibiting double the silicon concentration compared to Group 2. While other elements such as titanium, carbon, and oxygen were present in similar concentrations across both groups.

Group 2 shows minor sodium presence. No bacterial colonization is observed across all groups, indicating the efficacy of biomaterial impregnation.

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