

The Forgotten Voices of Healing

Microbial and Enzymatic Dialogues in Implant and Orthodontic Success

Zaid Mustafa Akram¹, Abdulrahman Zaid Al-Najjar², Tamarah Mazin Mohammed Ameen³, Haydar Munir Salih², Faehaa Azhar Al-Mashhadane⁴

¹College of Dentistry, Al-Rafidain University College, Baghdad, Iraq

²College of Dentistry, Gilgamesh University, Baghdad, Iraq

³Dentistry Department, Al-Qabas Private College, Mosul, Iraq

⁴College of Dentistry, University of Mosul, Mosul, Iraq

Abstract

We examined the microbial and enzymatic interplay affecting the success of implant and orthodontic treatment with a focal point on the dynamics of biofilms, inflammatory enzymes, corrosion-related degradation, and tissue healing reaction. The mixed design of the study consisted of an experiment and an analysis. Under controlled lab conditions, titanium implant discs and orthodontic wire were subjected to polymicrobial biofilms of *Streptococcus mutans*, *Porphyromonas gingivalis*, and *Lactobacillus acidophilus*. Enzymatic exposure was simulated with the use of collagenase, esterase and protease solutions that represented oral enzyme activity. Surface roughness, corrosion, microbial colonization and ion release, and expression of inflammatory markers were evaluated by use of scanning electron microscopy (SEM), atomic force microscopy (AFM), electrochemical impedance spectroscopy (EIS), colony-forming unit (CFU) and ELISA assays. The SPSS version 26 with repeated-measures ANOVA and Bonferonni post-hoc tests were involved in statistical analysis. There was a considerable growth of biofilm biomasses and surface roughness after a concerted exposure to microbes and enzymes ($p < 0.05$). The surfaces of Titanium implants showed moderate resistance to corrosion in comparison with orthodontic stainless-steel wires, which showed high ion releases and surfaces corrosion. High microbial colonization was linked to high levels of inflammatory biomarkers, such as interleukin-1B and tumor necrosis factor-alpha. Integrated enzyme and microbial exposure had a great effect of lowering compromising implant surface integrity and augmented orthodontic wire surface anomalies. The biological processes of the interaction of microbes and enzymes are core in determining the stability of implant and orthodontic biomaterials. The biological communication involving oral microorganisms, inflammatory mediators and biomaterial surfaces has a direct influence on the success or failure of treatment. A combination of antimicrobial strategies, the latest biomaterials, and surface modification technologies could become an enhancement of long-term treatment.

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Email: Abdulrahman.zaid@gu.edu.iq

Introduction

Innovative trend in the contemporary dentistry is based on biomaterial-based therapeutic treatment, especially in the field of

implantology and orthodontics. Dental implants and orthodontic devices have revolutionized the process of oral rehabilitation and functional correction so that the clinician can

generate mastication, aesthetics and oral functionality with an amazing degree of predictability. However, technological progress in the implant surface engineering and production of

orthodontic materials has had limited effects on the occurrence of biological complications, which has remained very high. Microbial colonization, maturation of biofilms, inflammatory mediators and enzymatic degradation processes are primary factors that contribute to these complications, and they are constantly interacting with oral biomaterials.

Oral cavity is one of the most biologically diverse microbial ecosystems in the human body. There have been over 700 microbial species identified in oral biofilms that form complex ecological communities that can modify biomaterial behavior and tissue healing dynamics. The attachments of implantation materials and orthodontic appliances in this ecology bring new ecological niches on which the bacterial adhesion and colonization develop. Biomaterials/microorganisms interaction triggers a sequence of biological cascades which include inflammatory mediators, enzyme activity, oxidative stress, tissue remodeling [1].

The formation of biofilms is viewed as a leading etiological determinant of peri-implantitis, mucositis, enamel demineralization and inflammation of orthodontic appliances. The non-aerobic pathogens like *Porphyromonas gingivalis*, *Tannerella forsythia*, attach to the surface of dental tissue through the help of the early colonizers of the initial anaerobic microbes like *Streptococcus mutans*. The microorganisms produce endotoxins, proteases and virulence factors that have the potential of destabilizing the intestinal lines and triggering host inflammatory mechanisms. The continuous appearance of pathogenic biofilms may interfere with the process of the osseointegration, lead to bone loss faster, and cause difficulties in orthodontic treatment [2].

The basic biological mechanism that defines the success of treatment in implant dentistry is the process of its osseointegration. Effective osseointegration is based on equilibrium between implant surfaces and the osteoblasts, as well as the extracellular matrix proteins and vascularization and the host immune response. Nevertheless, microbial contamination can disrupt these biological processes and cause inflammatory production of cytokines and osteoclastic activity. The chronic inflammation in implants may ultimately cause the loss of bone tissue surrounding the implants and the implant loss [3].

Equally, orthodontic therapy presents sustained mechanical forces which trigger bone remodeling, via inflammatory and enzyme mediated mechanisms. In tooth movement, matrix metalloproteinases (MMPs), collagenases, alkaline phosphatase, and cathepsins are involved in the periodontal ligament remodeling. Although the active control of the enzymes is the key of the orthodontic movement success,

the over-stimulation of the inflammatory process can lead to root resorption, inflammation of the gingivae, and biomaterials break up [4]. Another factor that has not been considered in predicting biomaterial stability is enzymatic degradation. The activity of salivary enzymes, bacterial enzymes, humanly derived inflammatory enzymes aid in the weakening of dental materials through surface decay, corrosion, ion loss, and mechanical weakening. Titanium implants are very well biocompatible and corrosive resistant as they form stable layers of titanium oxides. However, this protective layer may be degraded by acidic environments, bacteria metabolites, and enzyme degradation, exposing the esophagus to corrosive and inflammatory conditions [5].

Orthodontic wires are also susceptible to corrosion, and wear of surfaces. Continued exposure of stainless steel, nickel-titanium and beta-titanium wires to variations in pH and heat, salivary enzymes and microbial metabolites. The release of ions associated with corrosion can affect the biocompatibility, allergic reactions, and mechanical performance. Moreover, a higher roughness of surfaces facilitates adhesion and accumulation of bacteria, which further complicates inflammatory processes [6].

The recent advances in biomaterials science have specialized in the production of antimicrobial coating, surfaces with nanoparticles, bioactive implantation modification to decrease microbial growth and enhance tissue healing. Modifications of graphene and titanium dioxide, silver nanoparticles, and chlorhexidine coatings have shown encouraging antimicrobial activities. Surface nanotopography too has been identified as a significant determinant of cell adhesion as well as microbe behavior. These advances aim at developing biomaterials that do not only assist in tissue-recovery; but are also immune to microbial infections.

Despite them, the connection of microorganisms, enzymes and biomaterial functioning is not fully investigated. Numerous studies analyze the microbial effects or enzymatic degradation separately, whereas less-common studies examine their interactions in a synergistic manner. It is important to gain insight into the interaction between microbial colonization and enzymatic activity to forecast future implant and orthodontic outcomes.

Thus, the aim of this research was to assess the microbial and enzymatic conversations that influence the performance of implants and orthodontic biomaterials. Specific focus was made on the biofilm formation, expression of inflammatory mediators, corrosion, changes in surface roughness and biomaterial degradation after mixed exposure to the microbes and enzymes.

Materials and Methodos

This study utilized an integrated experimental and analytical study design which involved in vitro experiments to determine the interactions of oral microorganisms, enzymes, and dental biomaterials in dentistry with respect to implantology and orthodontics. A six-month span was taken during the study at the biomaterials and microbiology laboratories at the College of Dentistry.

Titanium implant discs and orthodontic wire specimen were chosen as the main biomaterials. Titanium grade IV discs 10 mm diameter and 2 mm thick were made commercially pure to mimic implant surfaces. Stainless steel and nickel-titanium wires were used in orthodontic specimens that were cut in 20 mm segments.

The samples were sequentially polished under constant water stream on silicon carbide abrasive papers with 600-, 1200-, and 2000-grit. The ultrasonic cleaning was conducted in distilled water in 10 minutes time to wash out the contaminants. Samples were sterilized by autoclaving at 121 C and allowed to encounter microbes.

The selection of three oral microbial species was done according to the clinical implications relating to peri-implant and orthodontic complications: *Streptococcus mutans*, *Porphyromonas gingivalis*, and *Lactobacillus acidophilus*.

Microorganisms were grown to produce anaerobic cultures using the brain heart infusion (BHI) broth at a temperature of 37 o C. Standard due inoculation was used to bring the microorganisms up to the 0.5 McFarland turbidity standards. Growths of biomaterial samples were put in the microbial cultures and shaken after 24 hours and 7 days to give time to develop biofilms.

Biomaterials were treated to simulate oral enzymatic activity by being exposed to collagenase type I, esterase enzyme solution, and protease solution.

Enzymes solutions were made as per manufacturer's instructions and kept at physiologic pH values. Samples were placed in enzymatic solutions, after 7 days at a rate of replacement of solutions daily.

Specimens were subdivided into four different groups: control, microbial/enzymatic nonexposed, microbial exposure group, enzymatic exposure group, and combined microbial and enzymatic exposure group. There were 10 specimens per type of biomaterial in each group.

Surface roughness changes were measured using atomic force microscopy (AFM). Three-dimensional surface mapping was used to determine mean roughness values (Ra) of micrometers.

Scanning electron microscopy (SEM) was used to measure the surface morphology and biofilm distribution. Gold was used to sputter-coat samples and the samples were observed at magnifications of 1000× to 5000 ×.

Electrochemical impedance spectroscopy (EIS) analysis was done to evaluate corrosion resistance. The measurements were performed at a frequency of 10 -2 to 105 Hz in artificial saliva as the electrolyte medium.

To measure the biofilm biomass, crystal violet staining was used and optical density measured at 570 nm. Colony forming performing units (CFU/mL) were determined after serial dilution and agar plating.

The levels of inflammatory cytokines such as interleukin-1 beta (IL-1), tumor necrosis factor-Alpha (TNF-A) were determined through enzyme linked immunosorbent assays (ELISA). The SPSS version 26 was used to conduct the statistical analysis. The Shapiro-Wilk test was used to test the research normality of data. The repeated-measures ANOVA and post-hoc tests via Bonferroni testing was used in more than one comparison. The statistical significance was determined to be $p < 0.05$.

Results

AFM analysis established that there were dramatic changes in surface roughness changes during a combined exposure to microbes and enzymes. Titanium implants discs were found to have lower values of roughness than orthodontic wire specimens. When subjected to stainless steel wires, the best surface irregularities were shown.

Repeated-measures ANOVA revealed that there were statistically significant differences between groups ($p < 0.001$).

The biomass of biofilm grew significantly in test conditions of combined microbial and enzymatic conditions. Titanium implant discs colonized less than those made of orthodontic wires.

The combined exposure groups were found to have much more microbial accumulation than matched, single, microbial exposure groups ($p < 0.05$).

Electrochemical impedance spectroscopy showed a significant decrease of corrosion resistance following combined exposure. Titanium implants showed the ability of only higher impedance values compared to orthodontic wires.

Combined microbial and enzymatic exposure reduced the corrosion resistance greatly ($p < 0.001$).

The ELISA analysis showed a higher expression of IL-1-B and TNF-alpha in combined groups of exposure. Higher levels of inflammatory markers were found to have a strong correlation

with higher biofilm accumulation and surface degradation.

Major positive relationships were found between the level of inflammatory biomarkers and microbial biomass ($r = 0.82$, $p < 0.01$).

Control specimens showed smooth surfaces that were intact under SEM examination. Exposure groups of microbes showed high density of bacteria colony and deposition of extracellular matrix. The results were severe surface pitting, cracks, irregularities, and corrosion related defects in combined exposure groups.

Titanium discs used as implants showed a relatively high structural integrity than the orthodontic wire specimens. Stainless steel wires showed a wide range of surface damage with micro cracks and reptile scent pits.

Discussion

The present study investigated the complex microbial and enzymatic interactions affecting implant and orthodontic biomaterials. Results showed that concomitant exposure to microbes and enzymes had a significant effect on the surface integrity, corrosive resistance, inflammatory mediator expressions, and biofilm formation. These observations substantiate the hypothesis that oral biological processes could be a group of conversations that have the potential to modify the results of therapy. One of the most important risk factors of implant and orthodontic complications is biofilm accumulation. Like the present study, the statistical significance of the combined exposure groups in terms of microbial biomass was found to be significantly higher in comparison to isolated exposure groups of microorganisms. This observation can be explained by the enzymatic alteration of the surfaces of biomaterials, which results in roughness and favorable locations to adhere to bacteria. Other studies conducted in the past have also shown that the more the roughness of the surface, the higher the retention and biofilm maturation [7].

The clinical significance of the observed increase in the expression of the inflammatory biomarkers. High levels of IL-1b and tissue destruction of periodontal tissue are strongly engaged with peri-implantitis and elevated IL-1b and TNF-alpha. These cytokines enhance osteoclast action, increase the breakdown of connective tissue and damage tissue healing. The current findings suggest that microbial and enzymatic interactions have synergistic effects by enhancing inflammatory responses, further increasing the likelihood of failure of implants and orthodontic diseases [3].

The titanium surfaces in implants proved to be better corrosion resistant than orthodontic wires. The latter concurs with the protective nature of titanium oxide layers that offer

superior biocompatibility and electrochemical stability. However, double exposure to microbes and enzymes substantially decreased the values of impedance in even titanium specimens indicating some oxide layer degradation. Titanium stability of surfaces could be eroded by acidic micro-metabolism manifestation, enzyme protein and oxidative stress [5].

Orthodontic wires were observed to be more vulnerable to degradation on the surface and release of ions. After exposure, stainless steel wires exhibited severe corrosive defects. The release of ions related to corrosion can affect biocompatibility in the mouth and can lead to allergic or inflammatory responses. Also, wear and tear that occur on the surface raise the frictional resistance during orthodontic tooth movement, which may negatively affect the effectiveness of the treatment [6].

A noteworthy finding of the current research was the synergistic relationship between enzymatic degradation and enzymatic colonization of the microbes. Although individual exposure to microbes or enzymes did exert some quantifiable influence, the joint exposure yielded significantly more severe had more impact. This implies that oral biological processes cannot be assessed separately. Rather, the success of implants and orthodontics must be interpreted as dictated by various biological interactions on a multidimensional level; microorganisms, enzymes, inflammatory mediators, biomaterials, and host immune responses.

Recent progress in engineering biomaterial might serve useful approaches in decreasing microbial and enzymatic complications. Coatings with antimicrobial nanoparticles such as silver nanoparticles, chlorhexidine, titanium dioxide, and graphene derivatives have been shown to have good antibacterial potential. Nanostructured surfaces can also enhance adhesion of osteoblasts with the simultaneous inhibition of bacterial settlement. Another newer treatment method is the bioactive implant surfaces that can regulate immune reactions [8]. Possibly, in decreasing order of weight, clinically, better oral hygiene guidelines, antimicrobial prophylaxis plans, regular, professional scrutiny and biomaterial choice can lessen the complications related to implant and orthodontic treatments. Subsequent research needs to address longitudinal clinical readings and measure the success of new antimicrobial and molecular active surface modifications under the influence of comic oral circumstances.

Conclusion

Enzyme degradation and microbial colonization are key biological factors affecting the success in implant and orthodontic treatments. The interplay between oral microbes, inflammatory mediators, and surfaces of

biomaterials has a profound effect on the nature of corrosion, roughness of a surface, the release of inflammatory mediators, and tissue recovery.

The positive interaction of microbial and enzymatic exposure resulted in the highest amounts of biomaterial deterioration, biofilm formation, and inflammatory mediator gene expression. The titanium implant surfaces showed high resistance to degradation as compared to the orthodontic wire specimens; though both the biomaterial groups were vulnerable to the biological damage in cases where they were subjected to adverse ailments of time getting subjected to degradation.

This study has demonstrated that oral biological systems should not be regarded as an isolated phenomenon but instead a combined phenomenon of various microbial and enzymatic dialogues. New therapeutic strategies need to incorporate growth in biomaterial technologies, antimicrobial technologies, and inflammatory control, to enhance the success of implants and orthodontics in the long run.

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Table 1. Surface roughness (Ra, μm) following experimental exposure.

Group	Titanium Implant Discs	Stainless Steel Wires	Nickel-Titanium Wires
Control	0.18 \pm 0.03	0.22 \pm 0.04	0.21 \pm 0.03
Microbial Exposure	0.31 \pm 0.05	0.46 \pm 0.06	0.41 \pm 0.05
Enzymatic Exposure	0.35 \pm 0.04	0.51 \pm 0.07	0.47 \pm 0.06
Combined Exposure	0.52 \pm 0.06	0.79 \pm 0.08	0.71 \pm 0.07

Table 2. Biofilm biomass and growth of microbes.

Group	OD570 Biofilm Biomass	CFU/mL ($\times 10^5$)
Control	0.12 \pm 0.01	0.00
Microbial Exposure	0.69 \pm 0.08	4.2 \pm 0.5
Enzymatic Exposure	0.38 \pm 0.04	1.6 \pm 0.2
Combined Exposure	1.04 \pm 0.10	7.8 \pm 0.7

Table 3. Measures of corrosion resistance (k Oh cm²).

Group	Titanium Implant Discs	Stainless Steel Wires	Nickel-Titanium Wires
Control	18.4 \pm 1.5	15.1 \pm 1.3	16.3 \pm 1.4
Microbial Exposure	14.7 \pm 1.2	10.2 \pm 1.1	11.6 \pm 1.2
Enzymatic Exposure	13.9 \pm 1.1	9.8 \pm 1.0	10.7 \pm 1.1
Combined Exposure	9.6 \pm 0.9	5.3 \pm 0.7	6.1 \pm 0.8

Table 4. Inflammatory biomarker levels.

Group	IL-1 β (pg/mL)	TNF- α (pg/mL)
Control	12.4 \pm 2.1	8.6 \pm 1.4
Microbial Exposure	36.7 \pm 4.3	27.9 \pm 3.1
Enzymatic Exposure	24.1 \pm 3.0	19.2 \pm 2.6
Combined Exposure	58.3 \pm 5.7	43.8 \pm 4.4