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ASSESSMENT OF BACTERIAL ADHERENCE AND BIOFILM FORMATION OF STREPTOCOCCUS MUTANS AND PORPHYROMONAS GINGIVALIS ON XENOGRAFT AND PERIOGLAS BONE GRAFT MATERIALS – AN IN VITRO STUDY

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ABSTRACT

Background

Bacterial colonization and biofilm formation on bone graft materials can compromise periodontal regenerative outcomes. Xenografts and bioactive glass (PerioGlas®) are widely used graft materials; however, limited data exist regarding their susceptibility to microbial adherence.

Aim

To evaluate and compare the bacterial adherence and biofilm formation of Streptococcus mutans and Porphyromonas gingivalis on xenograft and PerioGlas® bone graft materials under in vitro conditions.

Materials And Methods

An in vitro study was conducted using xenograft and PerioGlas® samples inoculated with S. mutans and P. gingivalis. After incubation, biofilm formation was assessed using crystal violet staining. The intensity of staining was evaluated to quantify bacterial adherence and biofilm formation. All experiments were performed in triplicate.

Results

PerioGlas® demonstrated significantly reduced biofilm formation compared to xenograft. The percentage of biofilm inhibition by PerioGlas® was 19.64% for S. mutans and 36.1% for P. gingivalis, indicating superior resistance to bacterial colonization. P-values <0.05 were considered statistically significant.

Conclusion

PerioGlas® exhibited significantly lower bacterial adherence and biofilm formation than xenograft. These findings suggest that PerioGlas® may offer improved resistance to microbial colonization, potentially enhancing the success and predictability of periodontal regenerative procedures.

Keywords: Biofilm formation; Bacterial adherence; Xenograft; PerioGlas

INTRODUCTION

Periodontitis is a chronic inflammatory disease of the supporting structures of teeth caused by specific microorganisms, leading to progressive destruction of periodontal ligament and alveolar bone, ultimately resulting in tooth loss if untreated (1). The primary etiological factor in periodontal disease is microbial dental plaque organized as biofilms, which provoke an exaggerated host immune response, culminating in tissue breakdown and alveolar bone resorption (2). Epidemiological studies indicate that periodontitis affects a large proportion of adults worldwide and remains one of the leading causes of tooth loss (3).

The principal goal of periodontal therapy extends beyond infection control and inflammation reduction, aiming to regenerate the lost periodontal structures, particularly alveolar bone, cementum, and periodontal ligament (4). Regenerative procedures have become an integral component of modern periodontal therapy, especially in the management of intrabony defects, furcation involvements, and ridge augmentation procedures. Bone graft materials serve as scaffolds facilitating new bone formation and play a critical role in restoring lost periodontal support (5).

Bone graft materials are broadly categorized into autografts, allografts, xenografts, and alloplasts based on their origin (6). Xenografts, derived from animal sources, primarily bovine, are widely used due to their excellent osteoconductive properties, long-term volume stability, and favourable clinical outcomes. Bio-Oss® is one of the most commonly employed xenograft materials in periodontal regenerative procedures (7). Alloplastic grafts such as bioactive glass, including PerioGlas®, are synthetic materials composed primarily of calcium sodium phosphosilicate. These materials exhibit osteoconductive properties and are known for their bioactivity, stimulating osteoblastic differentiation and bone formation (8).

Despite their favourable regenerative potential, bone graft materials may be exposed to the oral environment during surgery or due to postoperative complications such as flap dehiscence or wound breakdown. This exposure renders graft materials susceptible to bacterial colonization and biofilm formation, potentially impairing healing, delaying regeneration, and increasing the risk of graft failure (9). Bacterial adherence to graft surfaces is the initial step in biofilm formation, followed by microbial proliferation and extracellular matrix production, creating a highly structured and resistant microbial community (10).

Among the diverse microbial species present in the oral cavity, Streptococcus mutans and Porphyromonas gingivalis represent key early and late colonizers, respectively. S. mutans plays a major role in supragingival plaque formation and early biofilm development, whereas P. gingivalis is considered a keystone pathogen in chronic periodontitis, capable of disrupting host immune responses and promoting tissue destruction (11). These microorganisms exhibit remarkable adhesive capabilities and biofilm-forming potential, making them ideal models for evaluating bacterial interaction with biomaterials.

Although numerous studies have investigated bacterial adherence on dental implants and barrier membranes, limited literature exists regarding the microbial colonization potential of periodontal bone graft materials. Understanding the interaction between commonly used graft materials and oral pathogens is essential for improving regenerative outcomes and developing infection-resistant biomaterials (12).

Therefore, this in vitro study was designed to evaluate and compare the bacterial adherence and biofilm formation of S. mutans and P. gingivalis on xenograft and PerioGlas® bone graft materials.

OBJECTIVES

To assess the initial bacterial adhesion of S. mutans and P. gingivalis on xenograft and PerioGlas® surfaces.
To visually compare the biofilm staining intensity using crystal violet assay.
To analyse differences in bacterial viability through colony-forming unit (CFU) counts or staining techniques.
To determine the relative resistance of the two graft materials to microbial colonization.

MATERIALS AND METHODS

Study Design

This was an in vitro experimental study conducted in collaboration between the Department of Periodontics, Sri Sankara Dental College, Varkala, and the Centre for Research on Molecular and Applied Sciences (P) Ltd.,

Trivandrum.
Materials Used
Osseograft - Xenograft bone graft granules
PerioGlas® bone graft granules
Bacterial strains: Streptococcus mutans and Porphyromonas gingivalis
Brain Heart Infusion (BHI) broth / Tryptic Soy Broth
Phosphate Buffered Saline (PBS)
Crystal violet stain (0.1% / 1%)
Ethanol (95%) / Acetic acid (30%)
Sterile test tubes
Study Groups
The samples were divided into four groups:
Group 1: Xenograft + Streptococcus mutans
Group 2: Xenograft + Porphyromonas gingivalis
Group 3: PerioGlas® + Streptococcus mutans
Group 4: PerioGlas® + Porphyromonas gingivalis
Each group contained two independent samples, and all procedures were conducted in triplicate.

METHODOLOGY

Preparation of Biofilm:
Standard strains of Streptococcus mutans and Porphyromonas gingivalis were cultured, and bacterial suspensions were adjusted to 0.5% McFarland standard. For S. mutans, nutrient broth was prepared and sterilized, while fluid thioglycollate medium was used for P. gingivalis. Twenty microliters of inoculum were added to sterile 96-well microtiter plates (for S. mutans) or PCR vials (for P. gingivalis). Plates and vials were incubated at 37°C for 72 hours under aerobic and anaerobic conditions respectively to allow biofilm formation.
Sample Treatment:
After 72 hours, biofilms were treated with 1–2 granules of the test samples (Perioglas and xenograft). Control wells were maintained without sample addition. The plates were further incubated for 24 hours.
Crystal Violet Assay:
Following incubation, wells were washed thrice with sterile distilled water to remove non-adherent cells and air-dried. Biofilms were stained with 1% crystal violet for 15–20 minutes, washed, and dried. The bound dye was solubilized using dimethyl sulphoxide (DMSO) or 30% acetic acid. Absorbance was measured at 570–600 nm using an ELISA plate reader to quantify biofilm formation and calculate percentage inhibition.

OUTCOME MEASURES

The outcome measures assessed were degree of bacterial adherence, biofilm formation intensity and percentage reduction in biofilm formation

STATISTICAL ANALYSIS

The collected data were statistically analysed using SPSS version 26.0. An independent t-test was applied to compare different organism types, while one-way ANOVA was used to assess variations under different growth conditions. Group differences were examined, and a p-value of less than 0.05 was considered statistically significant.

RESULTS

The present in vitro study evaluated and compared biofilm formation and bacterial adherence of Streptococcus mutans and Porphyromonas gingivalis on Xenograft and PerioGlas® bone graft materials under different experimental conditions.
Graph 1 shows the percentage inhibition between S. mutans and P. gingivalis showed no statistically significant difference ($p = 0.361$), although S. mutans demonstrated a relatively higher mean inhibition compared to P. gingivalis.
Graph 2 depicts the comparison between the two organisms which revealed no statistically significant difference in bacterial growth across all optical density (OD) measurements, including OD1, OD2, OD3, and average OD, with p-values of 0.880, 0.886, 0.921, and 0.977 respectively ($p > 0.05$).
Table 1 presents the comparison between P. gingivalis and S. mutans. The OD values (OD1, OD2, OD3, and average OD) show very high p-values (0.880, 0.886, 0.921, and 0.977), all of which are well above the standard significance threshold of 0.05, indicating that there is no statistically significant difference in bacterial growth between the two organisms.

This suggests that both species exhibited a similar growth pattern throughout the experiment. Furthermore, the percentage inhibition analysis yielded a p-value of 0.361, which is also greater than 0.05, indicating no statistically significant difference in the inhibitory effect of the materials on the two bacterial species. Although S. mutans demonstrated a higher mean inhibition (59.69%) compared to P. gingivalis (49.06%), this difference was not statistically significant, likely due to sample size and variability in the data.

Evaluation of different growth conditions, including aerobic, modified aerobic, and anaerobic environments, also showed no statistically significant differences in bacterial growth or percentage inhibition, with all p-values exceeding 0.05, indicating that environmental conditions did not significantly influence the outcomes.

Table 2 illustrates the comparison across different growth conditions, namely aerobic, modified aerobic, and anaerobic environments. The OD values and percentage inhibition were analysed using Welch's ANOVA, and all obtained p-values (0.698, 0.698, 0.574, 0.656, and 0.869) were well above the significance threshold of 0.05.

This indicates that there is no statistically significant difference in bacterial growth or in the effectiveness of the materials across the three growth conditions However, when observing the mean values, it can be noted that the modified aerobic condition consistently showed the lowest mean OD, while the anaerobic condition demonstrated the lowest mean percentage inhibition.

Although these patterns may suggest a possible trend, they are not statistically significant and therefore cannot be considered reliable based on the current dataset. In contrast, comparison between control and test materials demonstrated marked differences. The control groups for both organisms exhibited high optical density values, indicating maximum bacterial growth in the absence of graft materials. Both xenograft and PerioGlas® showed a reduction in OD values, confirming their antibacterial effect. Notably, PerioGlas® consistently demonstrated lower OD values compared to xenograft for both P. gingivalis and S. mutans, indicating reduced biofilm formation.

Table 3 compares the control, Perioglas, and Xenograft groups. The control groups for both P. gingivalis and S. mutans exhibited high mean OD values (0.439 and 0.487, respectively), indicating strong bacterial growth without inhibition. In contrast, both Perioglas and Xenograft showed markedly lower OD values, demonstrating significant antibacterial activity compared to the control.

Between the two test materials, Perioglas exhibited lower mean OD values (0.155 for P. gingivalis and 0.146 for S. mutans) than Xenograft (0.292 and 0.246, respectively), indicating reduced bacterial growth. Correspondingly, Perioglas also showed higher percentage inhibition (64.3% and 70.1%) compared to Xenograft (33.8% and 49.3%), confirming its greater antibacterial effectiveness. The results suggest that PerioGlas® is more effective than xenograft in reducing bacterial adherence, although no statistically significant differences were observed between bacterial species or growth conditions.

The findings of this study support that PerioGlas® has greater resistance to bacterial colonization than xenograft, making it a potentially more favourable material for periodontal regenerative procedures

DISCUSSION

Successful periodontal regeneration depends on optimal wound healing, stability of the graft material, and resistance to microbial contamination. Bacterial colonization of graft surfaces can compromise healing, disrupt clot stability, and impair new attachment formation (13). Hence, understanding the microbial interaction with graft materials is crucial for optimizing regenerative outcomes.

The present study evaluated bacterial adherence and biofilm formation of S. mutans and P. gingivalis on xenograft and PerioGlas® materials. The results demonstrated significantly lower biofilm formation on PerioGlas® compared to xenograft. PerioGlas® reduced biofilm formation by 19.64% for S. mutans and 36.1% for P. gingivalis, indicating superior antimicrobial resistance These findings are consistent with the observations of Stoor et al. who demonstrated antibacterial activity of bioactive glass against several oral microorganisms. The antimicrobial effect of bioactive glass is attributed to its ionic dissolution, which increases local pH and osmotic pressure, creating an unfavourable environment for bacterial survival. Additionally, the release of calcium and phosphate ions promotes remineralization and enhances tissue healing.

In contrast, xenografts, although highly osteoconductive, lack inherent antimicrobial properties and may serve as a substrate for bacterial colonization due to their porous structure. Wang et al. [9] reported significant bacterial adherence to collagen membranes, supporting the notion that biomaterial surface characteristics strongly influence microbial attachment. Surface roughness, porosity, and surface energy are key determinants of bacterial adhesion (14).

P. gingivalis exhibited higher susceptibility to inhibition by PerioGlas® compared to S. mutans, which may be explained by its anaerobic nature and sensitivity to alkaline environments. The alkaline microenvironment produced by bioactive glass dissolution disrupts bacterial membrane integrity and enzyme function, leading to

reduced viability (15).

The clinical implications of these findings are significant. In periodontal regenerative procedures, early bacterial contamination is a major risk factor for graft failure. The use of graft materials with intrinsic antimicrobial properties, such as PerioGlas®, may reduce postoperative infections and enhance regenerative success. Furthermore, these materials may decrease the dependence on systemic antibiotics, thereby limiting antibiotic resistance development.

The degree of bacterial colonisation is significantly influenced by the physicochemical properties of biomaterials. Microbial adhesion and the subsequent formation of biofilms are strongly influenced by surface topography, wettability, and surface free energy. By creating protected niches that enable early colonisation and biofilm structure maturation, materials with uneven surfaces and higher surface roughness typically encourage bacterial retention. The first stage of microbial adherence is determined by the interaction between bacterial cell walls and biomaterial surfaces, which eventually affects the course of periodontal infection, according to studies by Subramani et al..

PerioGlas® and other bioactive glass materials have special biological characteristics that enhance their antibacterial efficacy. When bioactive glass comes into touch with bodily fluids, it experiences surface reactions that lead to the precipitation of hydroxycarbonate apatite and the creation of a layer rich in silica. In addition to strengthening the link with host tissues, this reaction causes ionic exchange mechanisms that raise local pH and osmotic pressure, which prevents bacterial growth. The results of this investigation are corroborated by Allan et al.'s discovery that bioactive glass demonstrates broad-spectrum antibacterial activity against both gram-positive and gram-negative oral pathogens.

Bioactive glass has been demonstrated to promote cellular reactions that support periodontal regeneration in addition to its antibacterial qualities.

Bioactive glass releases calcium and phosphate ions that can promote osteoblast development, proliferation, and extracellular matrix synthesis. These ionic dissolution products have been reported to increase the regeneration potential of periodontal abnormalities by activating gene expression linked to bone formation (12). Consequently, a favourable environment for periodontal tissue healing and regeneration may be further enhanced by the decreased bacterial colonisation seen on PerioGlas® surfaces.

The composition and structural features of xenograft materials are another significant factor affecting bacterial adherence. Because of their osteoconductive qualities and structural resemblance to human bone, xenografts which are usually made from bovine bone are employed extensively. However, when exposed to the oral environment, their porous architecture and organic matrix remain may promote bacteria adhesion and biofilm buildup. Certain graft materials without intrinsic antimicrobial activity may serve as potential reservoirs for bacterial colonisation, especially in the early stages of wound healing, according to a study conducted by Al-Rasheed et al.

Furthermore, because biofilms show enhanced resistance to host immune responses and antimicrobial treatments, biofilm formation on graft materials is therapeutically significant. Biofilms can cause persistent inflammation and delayed healing once they are developed on biomaterial surfaces. According to Marsh et al oral biofilms are more challenging to remove than planktonic bacteria because they are highly ordered microbial communities with synergistic interactions. In order to avoid early microbial contamination and increase the predictability of periodontal regenerative operations, it may be essential to choose graft materials that are naturally resistant to bacterial adhesion.

However, this study has limitations inherent to in vitro models. The oral environment is complex, involving saliva, host immune factors, mechanical forces, and multispecies biofilms, which cannot be fully simulated in laboratory conditions. Future in vivo studies and clinical trials are warranted to validate these findings under clinical conditions.

CONCLUSION

Within the limitations of this in vitro study, both xenograft and PerioGlas® demonstrated antibacterial activity against Streptococcus mutans and Porphyromonas gingivalis. However, PerioGlas® consistently exhibited superior resistance to bacterial adherence and biofilm formation, as evidenced by lower optical density values and higher percentage inhibition.

Further in vivo studies and clinical trials are recommended to validate these findings under physiological conditions.

CONFLICT OF INTEREST

The author declares that there is no conflict of interest regarding the publication of this study.

FUNDING

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ETHICAL APPROVAL

This study was conducted entirely in vitro and did not involve human participants, animal subjects, or human/animal-derived cells; therefore, ethical approval was not required.

DATA AVAILABILITY

The authors confirm that the raw data that support the finding are available from the corresponding author upon request.

AUTHOR CONTRIBUTIONS (CREDIT STATEMENT)

Dr Sapna Mariyam Jacob : Conceptualization, Methodology, Investigation, Data curation, Formal analysis, Writing – original draft.

Dr Mathew John [Guide]: Conceptualization, Supervision, Project administration, Writing – review & editing.

Dr Sabari ChandraMohan [Co-author 1]: Investigation, Data curation.

Dr Aswathy S [Co-author 2]: Investigation, Validation.

Dr Divya Soman [Co-author 3]: Data curation, Formal analysis, Visualization.

Dr Vinitha Nair [Co-author 4]: Methodology, Resources, Writing – review & editing.

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LIST OF FIGURES AND TABLES

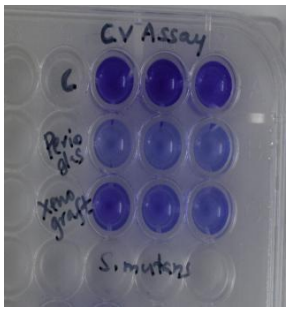


Fig 1a : CV assay of Streptococcus mutans

Fig 1b : CV assay of Porphyromonas gingivalis

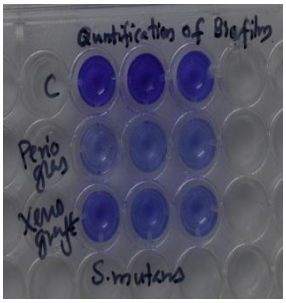


Fig 2a: Quantification of Streptococcus mutans

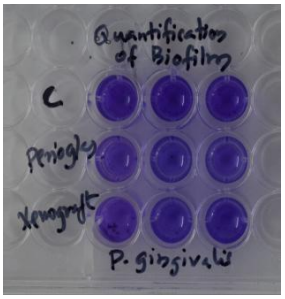
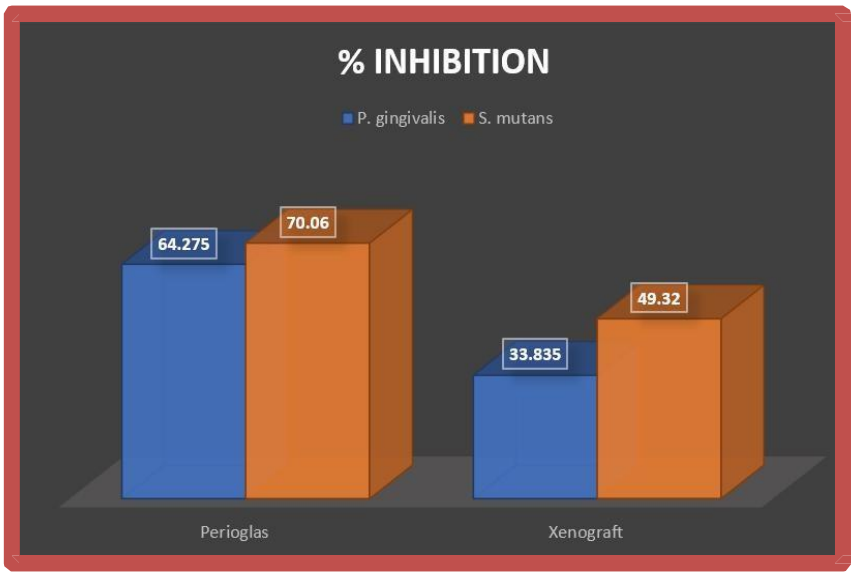


Fig 2b: Quantification of Porphyromonas gingivalis

GRAPH 1: Antibacterial activity (Percentage of Inhibition) of Perioglas and Xenograft against Porphyromonas gingivalis and Streptococcus mutans



GRAPH 2: Comparison of Mean Optical density of Porphyromonas gingivalis and Streptococcus mutans

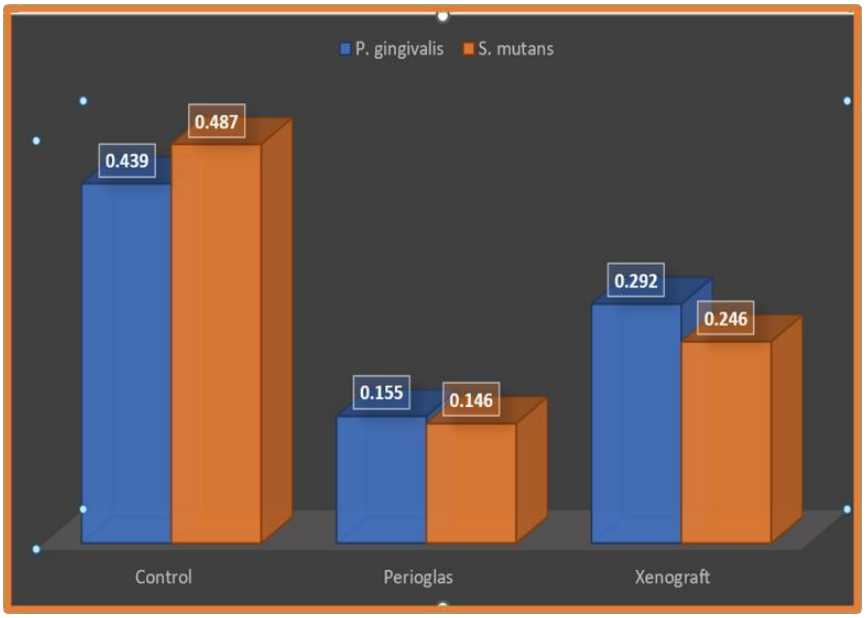


Table 1: Descriptive Statistics and Independent T-Test for Organism Type (P. gingivalis vs. S. mutans)

Group	**N**	**Mean**	**Median**	**SD**	**SE**	**p-value**
OD 1						
P. gingivalis	6	0.282	0.283	0.140	0.0570	0.880
S. mutans	6	0.296	0.234	0.175	0.0716	
OD 2						
P. gingivalis	6	0.292	0.275	0.138	0.0565	0.886
S. mutans	6	0.279	0.218	0.163	0.0667	
OD 3						
P. gingivalis	6	0.312	0.316	0.136	0.0554	0.921
S. mutans	6	0.303	0.285	0.177	0.0721	
Average OD						
P. gingivalis	6	0.295	0.292	0.137	0.0560	0.977
S. mutans	6	0.293	0.246	0.171	0.0697	
% Inhibition						
P. gingivalis	4	49.055	49.150	17.876	8.9381	0.361
S. mutans	4	59.690	60.240	12.010	6.0049	

Table 2: Descriptive Statistics and One-Way ANOVA for Growth Condition (Aerobic, Aerobic(Modified), Anaerobic)

Condition	**N**	**Mean**	**SD**	**SE**	**p-value**
OD 1					
Aerobic	3	0.348	0.207	0.1193	0.698
Aerobic(Modified)	6	0.244	0.129	0.0526	
Anaerobic	3	0.321	0.170	0.0982	
OD 2					
Aerobic	3	0.329	0.202	0.1166	0.698
Aerobic(Modified)	6	0.242	0.112	0.0456	

Anaerobic	3	0.329	0.177	0.1022	
OD 3					
Aerobic	3	0.364	0.223	0.1290	0.574
Aerobic(Modified)	6	0.253	0.111	0.0453	
Anaerobic	3	0.361	0.159	0.0918	
Average OD					
Aerobic	3	0.347	0.210	0.1212	0.656
Aerobic(Modified)	6	0.246	0.116	0.0474	
Anaerobic	3	0.337	0.168	0.0969	
% Inhibition					
Aerobic	2	60.270	13.888	9.8200	0.869
Aerobic(Modified)	4	54.130	14.660	7.3298	
Anaerobic	2	48.960	25.527	18.0500	

Table 3: Descriptive Statistics by Organism and Sample Type (Control, Perioglas, Xenograft)

Organism	**Sample**	**N**	**Mean**	**Median**	**SD**	**Minimum**	**Maximum**
P. gingivalis							
	Control	2	0.439	0.439	0.087	0.378	0.500
	Perioglas	2	0.155	0.155	0.014	0.145	0.165
	Xenograft	2	0.292	0.292	0.075	0.239	0.345
S. mutans							
	Control	2	0.487	0.487	0.132	0.393	0.580
	Perioglas	2	0.146	0.146	0.039	0.118	0.173
	Xenograft	2	0.246	0.246	0.059	0.204	0.287
% Inhibition							
P. gingivalis							
	Perioglas	2	64.275	64.275	3.868	61.540	67.010
	Xenograft	2	33.835	33.835	4.137	30.910	36.760
S. mutans							
	Perioglas	2	70.060	70.060	0.042	70.030	70.090
	Xenograft	2	49.320	49.320	1.598	48.190	50.450