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Efficacy of Hydrochloric Acid on The Removal of Accumulated Deposits on Reused Dental Implant Gingival Former Prior to the Sterilization Process A (Case-Control Analysis) Study

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Abstract

Reusing dental implant gingival formers has become common to reduce treatment costs, but remnant biofilm biomass on these reused gingival formers can promote infection. Recent studies have found that existing cleaning and sterilization methods fail to completely eliminate biofilm. This study introduces a novel approach for cleaning and sterilizing gingival formers, significantly improving debris removal and potentially reducing the risk of infection, thereby enhancing patient safety and treatment outcomes. To assess the efficacy of hydrochloric acid solution in removing accumulated debris and sterilizing previously used dental implant gingival formers.

Material and method: Forty new dental implant gingival formers were placed in patients' mouths for at least 3 weeks. They were then cleaned using two different methods: one with varying concentrations and durations of hydrochloric acid solution (HCl), and one without HCl. After cleaning and sterilization, the formers underwent Scanning electron microscopy (SEM) and microbiologic culture analysis.

Results: Treatment with a hydrochloric acid solution (HCl) in the concentration of 0.05 mol/L for 10 minutes appears to have the most favorable outcomes in terms of reducing implant remnant and less surface damage.

Conclusion: The application of hydrochloric acid solution (HCl) showed significant effectiveness in removing debris from the surface of used dental implant gingival formers. However, complete removal and thorough cleaning of debris remnants were not achieved, suggesting that reusing dental implant gingival formers may not be advisable.

Introduction:

Reusable medical devices are items that medical professionals can reprocess and use on multiple patients, according to the United States Food and Drug Administration (1). There are two ways that reusable medical devices can be used: Ad-hoc (used for a specific purpose before reprocessing and reusing on another patient) or in situ (left in place in one patient's body for a certain amount of time before being taken out, cleaned, and used on another patient) (2). In dentistry, several instruments are used repeatedly when treating the same patients across all dental specialties. These include surgical tools, burs, bone saws, endodontic files, periodontal tools, orthodontic tools, x-ray holders, and several instruments for pediatric dentistry. Common examples of ad-hoc reusable devices in implant dentistry include surgical instruments, surgical handpieces, surgical drills, bone-cutting burs, impression copings, dental implant gingival formers, and several other items (3). The dental implant gingival formers are made to accomplish several tasks: It protects the internal aspect (usually a screw thread) within the implant body from the impaction of debris during the osseointegration healing phase, also when composed of a biocompatible material such as titanium or titanium alloys it can support and allow the spread of the nonbony superficial soft tissues during healing, then maturation (4). The gingival formers are eventually removed and replaced with a final abutment and prosthesis once the implant has sufficiently matured the soft tissues and osseointegration has been clinically confirmed. Gingival formers are generally designated by the manufacturer for single use (5), also it is common practice that many clinicians clean and sterilize this component, often re-using it for economic reasons (6). Additionally, some companies collect these used parts, clean, sterilize, and repackage them for sale (7). Studies have shown that titanium gingival formers can be sterilized successfully (8). Additionally, the method of sterilizing may occasionally increase soft tissue cell adherence and spread along a clean titanium surface (9). Recent research, however, has raised concerns about the safety of reusing some of these components because they may not be as sterile or clean as originally believed (10). Numerous substances, including saliva, epithelium cells, food particles, and blood, can contaminate gingival formers components. The high adhesion of proteins and amino acids makes it difficult to adequately clean contaminated titanium surfaces, despite the development of specific cleaning processes (11). Additional biological and mechanical effects could happen if the re-used gingival formers have residual substance on surfaces other than those in direct contact with the healing soft tissues. These are related to the location of the contaminated residue. For example, debris accumulation at the Implant Abutment Junction (IAJ) may prevent the components from fitting correctly (4). For the implant abutment joint to mechanically function, a clean screw thread is essential. Changes could have an impact on the screw's friction as it is torqued to create the proposed preload for the final location of the abutment (12). Unsterile surfaces of gingival formers can result in inflammation of the peri-implant mucosal cuff and impede smooth healing processes(13).This study aims to determine if the hydrochloric acid solution (HCl) can be used to completely clean and sterilize dental implant gingival formers without causing harm to the surface and determine the effectiveness of sterilization in terms of microorganism survival.

Material and Method:

In this study, 42 non-used dental implant gingival formers from OXY dental implants by Biomec S.R.L were used. 40 of these gingival formers were distributed in 2 different clinics in Halabja and Sulaymanih/ Kurdistan in 2023 to be placed in patients' mouths for a period not less than three weeks. After the collection of the gingival formers from the clinics, the cleaning and sterilization process started as follows:

The 42 gingival formers were divided into a control group consisting of 2 unused

gingival formers and a study group consisting of 40 gingival formers that were used in the patient's mouth. As the gingival formers from the control group were already new and unused they were sterilized by the manufacturer no cleaning process was performed on them. For the gingival formers of the study group, two different methods of cleaning were used, first, the routine method of cleaning that is performed in most dental clinics, in this method four of the gingival formers were used, the gingival formers were washed with water, and soup and then placed in an ultrasonic bath for 15 minutes and after removal from the ultrasonic bath the gingival formers were dried with sterilized gauze and placed in pouches to be placed in a Class B autoclave in a program for metals. The second method was the special method in which hydrochloric acid solution (HCl) was used in the cleaning of the gingival formers. The hydrochloric acid (HCl) solution was prepared at three different concentrations which were 0.03 mol/L, 0.05 mol/L, and 0.1 mol/L, as shown in Figure (1).

The 36 remaining used gingival formers were divided into 3 groups, 12 gingival formers for each group.

This means each 12 gingival formers for one specific concentration of the (HCl) solution. The 12 gingival formers were further divided into another 3 groups, 4 gingival formers for each group, as shown in Figure (2). The gingival formers were placed in each concentration of (HCl) solution for different periods which were 5 min, 10 min, and 15 minutes. This means that 4 gingival formers were placed in an (HCl) concentration of 0.03 mol/L for 5 minutes another 4 gingival formers in the same concentration for 10 minutes and another 4 gingival formers in the same concentration for 15 minutes.

After removal from the (HCl) solution, the gingival formers were placed in an ultrasonic bath for 15 minutes, as shown in Figure (3). After removal from the ultrasonic bath, the gingival formers were dried with sterilized gauze and placed in pouches to be placed in a Class B autoclave in a program for metals. The summary of the method is depicted in the as shown in Figure (4):

After the cleaning and sterilization process, half of the samples were sent for Scanning Electron Microscopy (SEM), and the other half for microbiological culture.

Microbiologic Culture:

The gingival formers were removed from the pouches and placed into test tubes filled with a nutrient-rich microbiological solution (10 mL of Brain Heart Infusion broth (BHI)), as shown in Figure (5). Following stringent aseptic procedures. These tubes were then kept in an incubator at 37°C with a 5% CO2 environment for 10 days. Visual inspections were carried out daily to observe any signs of cloudiness in the solution. After the experiment concluded, the tubes were reexamined, and the contents were transferred onto Petri dishes containing BHI agar (BHIA) for further analysis, as shown in Figure (6).

Based on the inoculation on Brain Heart Infusion (BHI) broth and agar, no bacterial growth was observed. This statement indicates that no visible signs of bacterial activity, such as cloudiness in the broth or colonies on the agar plate, were detected after the incubation period. Indicating that the sterilization of the used abutments was completely satisfactory in terms of the removal of live bacteria or spores.

Scanning Electron Microscopy (SEM) analysis:

The scanning electron microscopy (SEM) images were captured at four points on the dental implant gingival formers as shown in Figure (7), which were:

- Gingival former body (B)
- Screw thread/shank (S)
- Screwdriver engaging site (C)
- Top view screw engaging hole (H)

The determination of the debris remnant and damage on the surface of the gingival formers was conducted as follows:

We divided our formers into 3 parts:

- top view screw engaging hole (H).
- body of the gingival former and Connection to the implant site as one part (B, C).
- Screw thread shank and threads (S).

We assigned a percentage to the remnants and damages of the gingival formers, determined by comparing them to the control group gingival formers which were new and unused, additionally, we conducted comparisons among the study groups themselves.

Ultimately, a percentage was given to the overall debris remnant and damage on each sample within every group.

Table (1), a unique code was assigned to each group of the gingival formers, ranging from BS 1 to BS 10. Each code represents a distinct combination of period and concentration of hydrochloric acid (HCl).

Statistical Analysis

The study employed descriptive statistical analysis to summarize the outcomes, providing mean and standard deviation for variables with continuous distributions. Variables were presented with proportion. Hypotheses regarding differences between two proportions were assessed using the Z score for proportions and differences between more than two proportions were assessed by Chi-square test for proportions. One sample t-test was used to assess differences between the sample and target value for those with a normal distribution. Before statistical analysis, the normal distribution assumption was confirmed using the Shapiro–Wilk and Kolmogorov–Smirnov tests. Statistical significance was determined with a threshold of a p-value less than or equal to 0.05. A p-value of 0.05 or lower was considered statistically significant in all tests. The analysis was conducted using version 27.0 of the SPSS program for Windows.

Results:

In this study, 42 non-used dental implant gingival formers sourced from OXY Dental Implant by Biomec S.R.L were inserted into patients' mouths for a minimum duration of three weeks. Following this period, the gingival formers underwent cleaning and sterilization using two distinct methods. One group was subjected to sterilization using hydrochloric acid (HCl), while the other group underwent the standard routine sterilization procedure. The study's outcomes are detailed below:

Analysis of debris remnant on the surface of dental implant gingival formers:

Table (2) presents the remnant percent of the debris in different groups, categorized by the type of treatment (HCl concentration) as shown in Figure (8). It also provides p-values indicating the statistical significance of the difference between the remnant percent in each group with a new implant and BS6 (No HCl treatment) using Z-scores for proportion. Based on the provided data, it appears that all three HCl concentrations (0.03, 0.05, and 0.1) significantly reduce the remnant percent of the debris compared to BS6 (No HCl treatment). This is evidenced by the very low p-values for all HCl concentrations, indicating a highly significant difference in remnant percent. Also, HCl concentration (0.03) significantly differs in the remnant percent of the implant compared to the new implant. However, HCl concentration (0.05 and 0.1) did not significantly differ in the remnant percent of the implant compared to the new implant.

Based on the provided data in Table 3, it appears that the remnant percent of the implant significantly differs from BS6 (No HCl treatment) at all three-time points (5, 10, and 15) as shown in Figure (9). This is evidenced by the very low p-values (<0.00001) for all time points, indicating a highly significant difference in remnant percent compared to BS6 (No HCl). However, the remnant percent of the implant does not significantly differ from

the new implant at all three-time points (5 and 10) because p-values are more than significant level 0.05.

Analysis of the amount of surface damage on the surface of dental implant gingival formers:

Table 4, the mean scores of surface damage significantly differ across different groups treated with varying HCl concentrations as shown in Figure (10). This is supported by the low p-values (0.001, 0.001, 0.002), indicating a significant difference compared to the new implant. The comparisons with a new implant suggest that treatment with HCl concentrations has a significant impact on surface damage compared to this benchmark.

Table 5, the mean scores of implant damage significantly differ across different time points as shown in Figure (11). This is supported by the low p-values (0.007, 0.001, 0.001), indicating a significant difference compared to a new implant. The comparisons with a new implant suggest that the mean score of surface damage changes significantly over time.

Finally, it can be concluded that treatment with a hydrochloric acid solution (HCl) in the concentration of 0.05 mol/L for 10 minutes appears to have the most favorable outcomes in terms of reducing implant remnant and less surface damage. Treatment with an HCl in the concentration of 0.05 mol/L for 10 minutes results in a relatively low remnant percent of debris on the surface of dental implant gingival formers, suggesting effective removal or dissolution of accumulated deposits. Additionally, the mean score of surface damage associated with treatment using HCl concentration 0.05 mol/L for 10 minutes is comparatively low, indicating minimal damage to the implant material during the treatment process. Therefore, considering both factors of remnant percent and surface damage, treatment with HCl concentration 0.05 for 10 minutes is likely to provide the best overall effect in reducing debris remnants while minimizing surface damage.

Discussion:

In this study, the focus was on utilizing hydrochloric acid solution (HCl) for the removal of accumulated debris on the surface of dental implant gingival formers. HCl solution was applied at three different concentrations for three distinct periods. Hydrochloric acid (HCl) is a strong, colorless, highly corrosive mineral acid It's known for its pungent odor and its ability to dissolve many metals and forms of minerals(14). So very low concentrations of the acid which were (0.03, 0.05, 0.1 mol/L) were used. Non-used dental implant gingival formers were placed in the patient's oral cavities after collecting them the cleaning and sterilization process started. Gingival formers are generally designated by the manufacturer for single use (15), also it is common practice that many clinicians clean and sterilize this component, often re-using it for economic reasons(6). Additionally, some companies collect these used parts, clean, sterilize, and repackage them for sale (7). Recent research, however, has raised concerns about the safety of reusing some of these components because they may not be as sterile or clean as originally believed (10). Wadhwani et al. in 2016 conducted a study (In-Vitro Study of the Contamination Remaining on Used Healing Abutments after Cleaning and Sterilizing in Dental Practice). 100 gingival formers were used in this study The method of sterilization and cleaning was Mechanical wiping with disinfection cloths, ultrasonic bath for between 10 and 60 minutes in various solutions, some used water, and some alcohol. All HAs were steam autoclaved. In the result of the study, 99% of the abutments showed protein contamination at one or more sites following cleaning and sterilization (16). Sanchez-Garces et al. in 2019 investigated if the re-use of sterilized implant gingival formers is safe enough. In his study, a total of 55 gingival formers previously used in one or more patients were used. The method of cleaning and sterilization used was: gingival formers were washed and sterilized in a steam autoclave at 121oC for 15 min. In the result of the study the

sterilization was completely satisfactory in terms of removal of live bacteria or spores Nevertheless, Significant amounts of the bioburden remained adhered to the surfaces despite the cleaning and sterilization procedures (17). Narvekar et al. in 2020 used four strategies available in clinical settings to determine if gingival formers can be “decontaminated” and compare the detoxification efficacy by quantifying residual biomaterial and capacity to elicit an inflammatory response in-vitro. The methods were: A: autoclave only, B: ultrasonic bath plus autoclave, C: prophy-jet plus autoclave, and D: Scrub sponge plus autoclave. In the result of the study, gingival formers were not entirely “decontaminated” using common methods available relative to new, sterile gingival formers and were capable of stimulating an immune response (18). Dental implant gingival formers play a crucial role as temporary elements in maintaining soft tissue and enhancing the aesthetic results of dental implants. They are usually removed after initial healing, sterilized, and then utilized again for multiple patients (5). It is generally known that many molecules, together with epithelial and blood cells, as well as bacterial fractions, may retain some properties following the sterilizing technique even when no living cells survive the autoclaving (19). The organic material that precipitated on the surface of reused abutments would come from the patient to whom the gingival formers were connected. It may then have an impact on cell adhesion, as well as the efficient spreading and attachment of epithelium and connective tissue (20). Additional biological and mechanical effects could happen if the re-used gingival formers have residual substance on surfaces other than those in direct contact with the healing soft tissues. These are related to the location of the contaminated residue. For example, debris accumulation at the Implant Abutment Junction (IAJ) may prevent the components from fitting correctly (4). For the implant abutment joint to mechanically function, a clean screw thread is essential. Changes could have an impact on the screw's friction as it is torqued to create the proposed preload for the final location of the abutment (12). In the results of this study in which HCl was used, it can be concluded that treatment with a hydrochloric acid solution (HCl) in the concentration of 0.05 mol/L for 10 minutes appears to have the most favorable outcomes in terms of reducing implant remnant and less surface damage. Treatment with an HCl in the concentration of 0.05 mol/L for 10 minutes results in a relatively low remnant percent of debris on the surface of dental implant gingival formers, suggesting effective removal or dissolution of accumulated deposits. Additionally, the mean score of surface damage associated with treatment using HCl concentration of 0.05 mol/L for 10 minutes is comparatively low, indicating minimal damage to the implant material during the treatment process. Therefore, considering both factors of remnant percent and surface damage, treatment with HCl concentration 0.05 for 10 minutes is likely to provide the best overall effect in reducing debris remnants while minimizing surface damage. The different methods of cleaning and sterilizing dental implant gingival formers utilized in this study were effective in reducing the amount of debris remnants but did not result in complete cleaning.

Conclusion:

Considering the limitations of this study, the findings indicate that hydrochloric acid solution (HCl) can effectively decrease debris on gingival formers' surfaces. However, it doesn't ensure complete removal or cleaning. Until additional evidence supports it, it's prudent to avoid indiscriminate reuse of gingival formers. Clinicians should assess each used healing abutment individually before considering reuse.

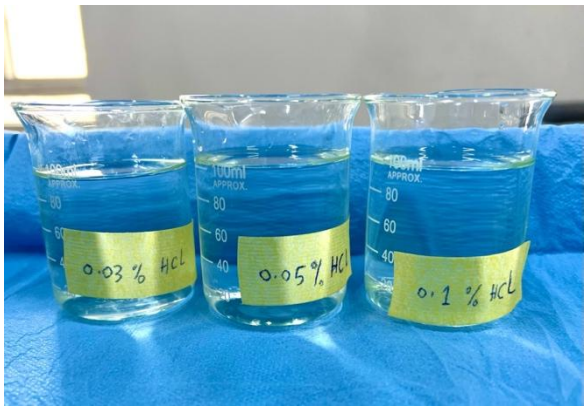

Figure 1: Different concentrations of HCl solution

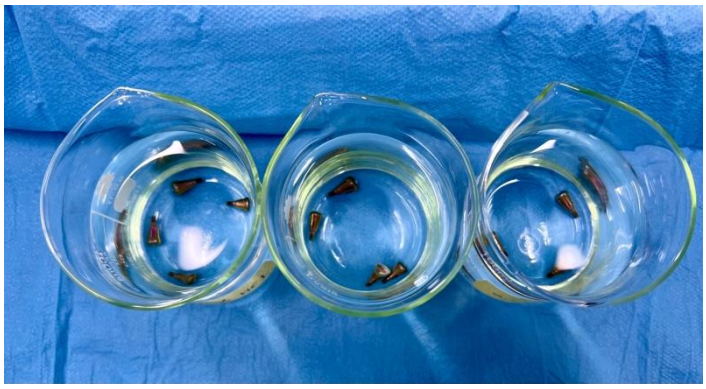

Figure 2: Four gingival formers in each different concentration of the HCl solution.

Figure 3: The gingival formers in an ultrasonic bath.

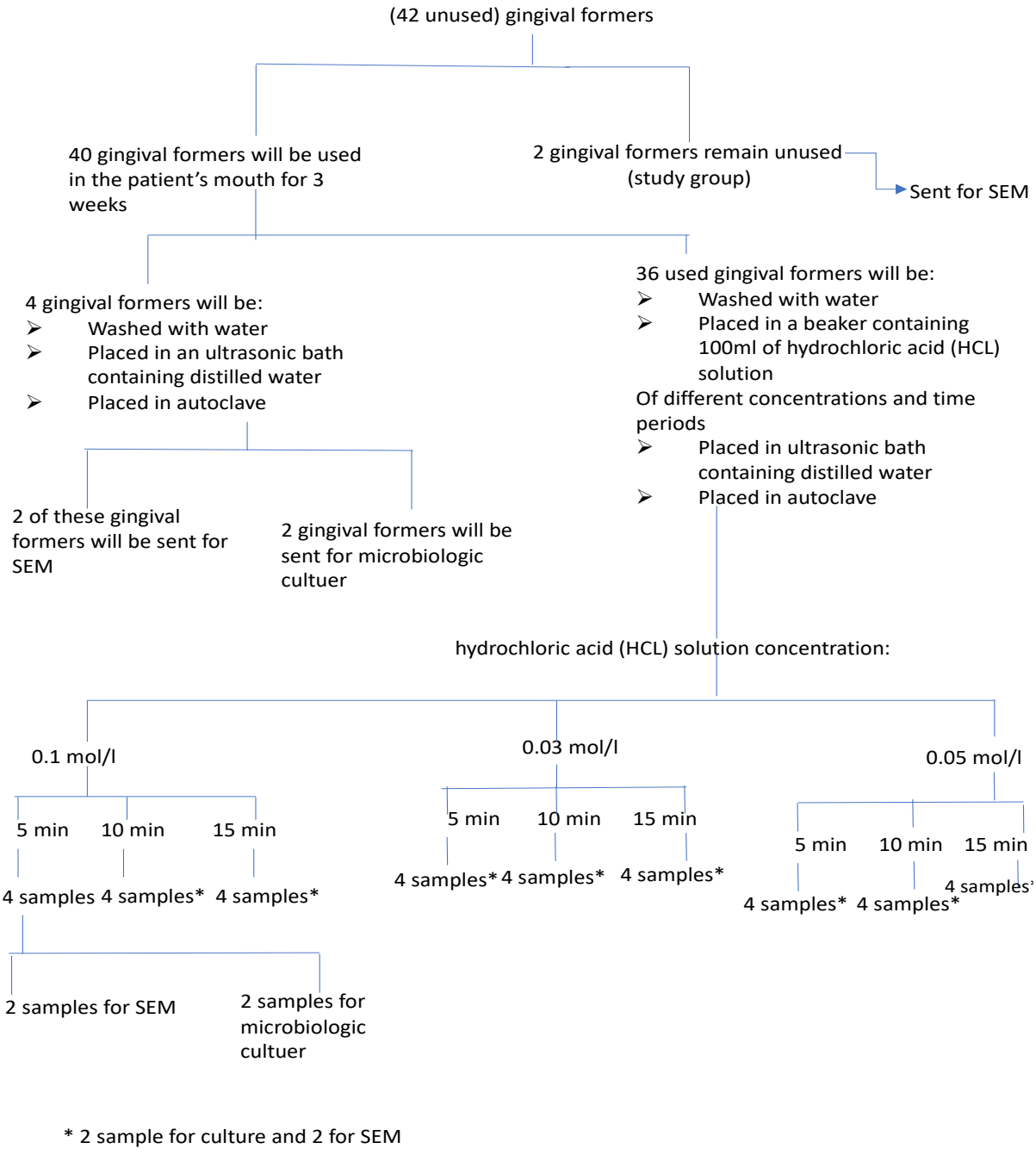

Figure 4: The summary of the method:

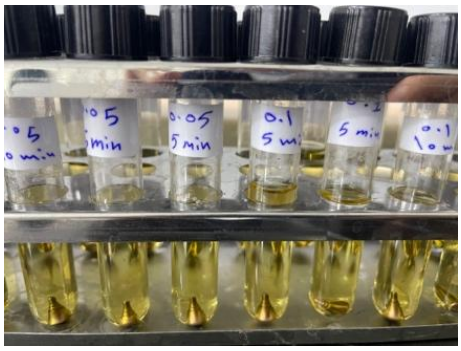

Figure 5: Microbiological cultures. tubes containing 10 ml of Brain Hearth broth, after incubation at 37°C for 10 days under a 5% CO2 atmosphere. No differences in turbidity were observed

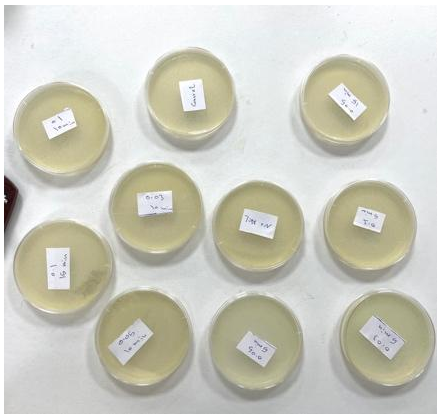

Figure 6: Petri dishes containing BHI agar.

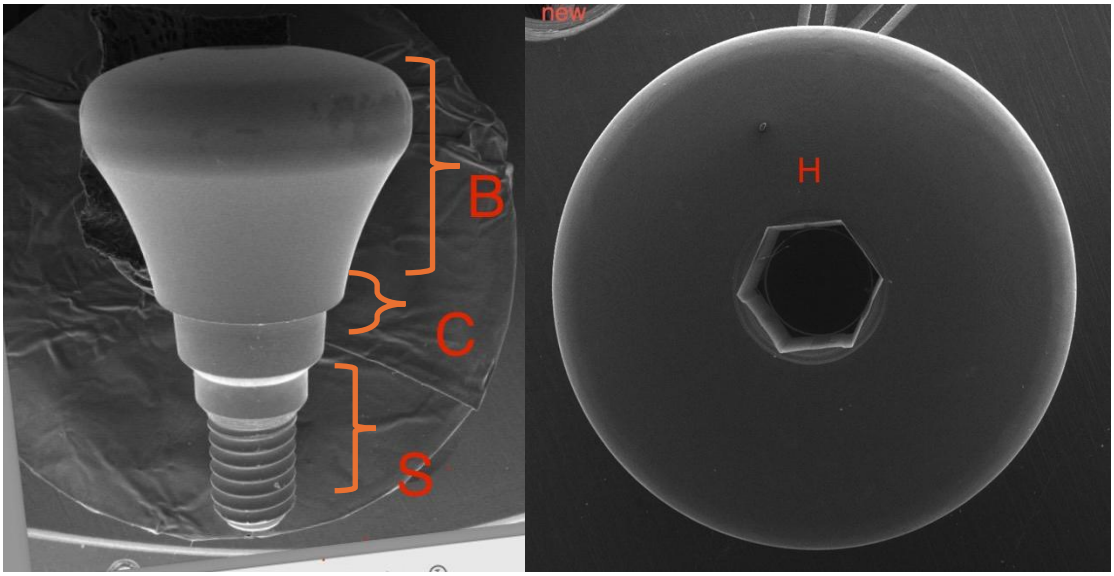

Figure 7: (B) body of the gingival former, (C) screwdriver connection site, (S) screw thread, (H) screw engaging hole.

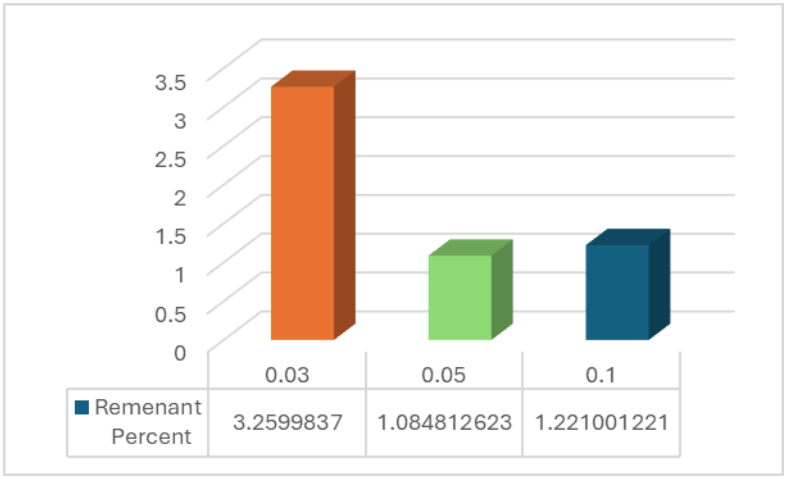

Figure 8. Remnant percent of debris in different groups of HCl concentration

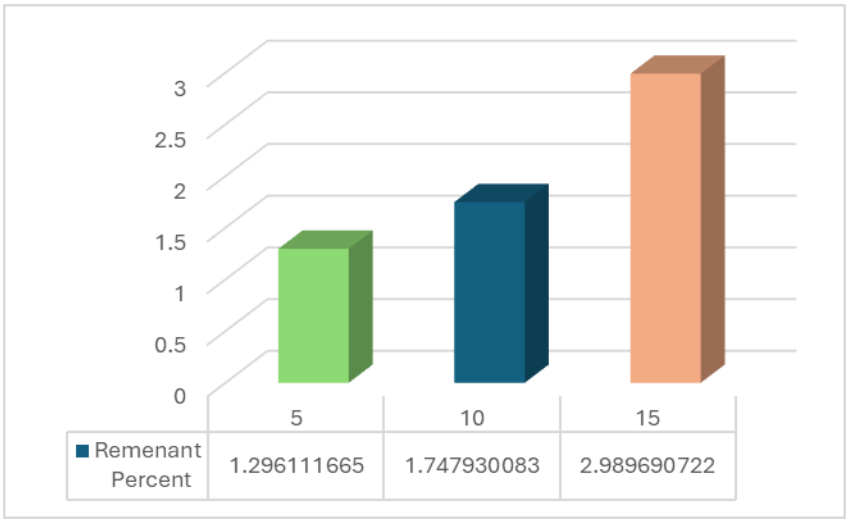

Figure 9. Remnant percent of debris at different times

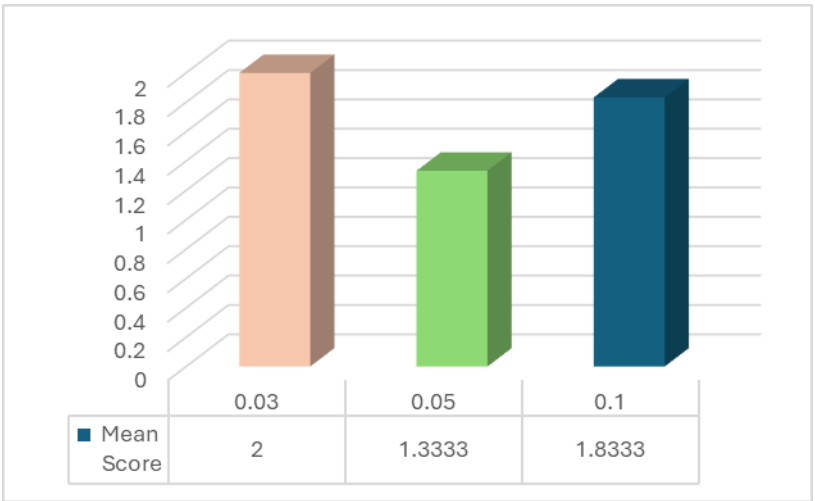

Figure 10. Mean score of surface damage in different groups of HCl concentration

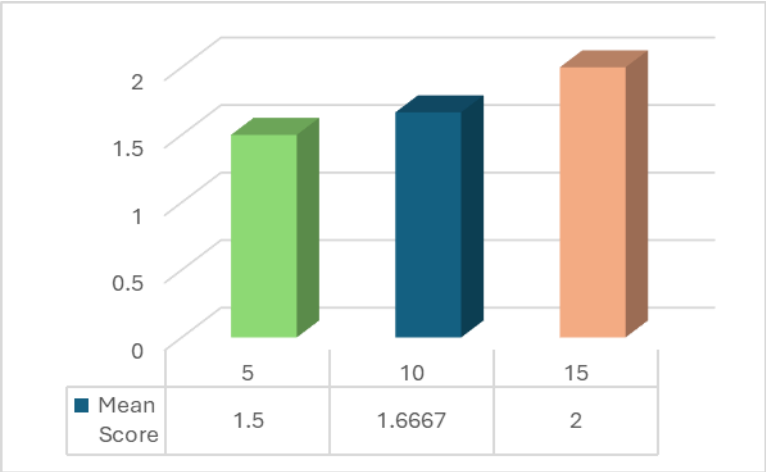

Figure 11. The mean score of surface damage at different times

Table 1: Codes of the gingival formers*:*

Code	(HCL) concentration	time
BS1	0.03 mol/L	10 minutes
BS2	0.03 mol/L	5 minutes
BS3	0.03 mol/L	15 minutes
BS4	0.05 mol/L	5 minutes
BS5	0.05 mol/L	15 minutes
BS6	NO HCl	
BS7	0.1 mol/L	15 minutes
BS8	0.1 mol/L	10 minutes
BS9	0.05 mol/L	10 minutes
BS10	0.1 mol/L	5 minutes

Table 2. Remnant percent of debris in different groups of HCl concentration

HCL	Remnant Percent	p-value [a]	p-value [b]
0.03	3.2599837	0.008	<0.00001
0.05	1.084812623	0.36	<0.00001
0.1	1.221001221	0.44	<0.00001

[a]: compare with the new implant by Z score for proportion
[b]: compare with BS6 (No HCl) by Z score for proportion

Table 3. Remnant percent of debris at different time periods

Time	Remnant Percent	P-value [a]	P-value [b]
5	1.29611167	0.35	<0.00001
10	1.74793008	0.15	<0.00001
15	2.98969072	0.01	<0.00001

[a]: compare with the new implant by Z score for proportion
[b]: compare with BS6 (No HCl) by Z score for proportion

Table 4. The mean score of surface damage in different groups of HCl concentration

HCL	Mean	SD	Se	p-value [a]	p-value [b]
0.03	2	0.63246	0.2582	0.001	0.111
0.05	1.3333	0.5164	0.21082	0.001	0.465
0.1	1.8333	0.75277	0.30732	0.002	0.328

[a]: compare with the new implant by One sample t-test
[b]: compare with BS6 (No HCl) by One sample t-test

Table 5. Mean score of surface damage at different time

Time	Mean	SD	Se	p-value [a]	p-value [b]
5	1.5	0.83666	0.34157	0.007	1
10	1.6667	0.5164	0.21082	0.001	0.465
15	2	0.63246	0.2582	0.001	0.111

[a]: compare with the new implant by One sample t-test
[b]: compare with BS6 (No HCl) by One sample t-test

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