ISSN: 2581-5989
PubMed - National Library of Medicine - ID: 101738774

International Journal of Dental Science and Innovative Research (IJDSIR)

IJDSIR : Dental Publication Service

Available Online at: www.ijdsir.com

Volume – 6, Issue – 5, September - 2023, Page No. : 157 - 167

To evaluate and compare the flexural strength of polyether ether ketone (PEEK) after evaluating the efficacy of different disinfection protocols on Candida albicans biofilm – An invitro study.

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Citation of this Article: Dr. Atreyee Deka, Dr. Bharat Raj R, Dr. Anupama N.M., Dr. Nalinakshamma M, Dr. Savitha P. Rao, "To evaluate and compare the flexural strength of polyether ether ketone (PEEK) after evaluating the efficacy of different disinfection protocols on Candida albicans biofilm – An invitro study.", IJDSIR- September - 2023, Volume – 6, Issue - 5, P. No. 157 – 167.

Type of Publication: Original Research Article

Conflicts of Interest: Nil

Abstract

AIM: To evaluate and compare the flexural strength of polyether ether ketone (PEEK) after evaluating the efficacy of different disinfection protocols on Candida albicans biofilm – an invitro study.

Method: A total of 60 PEEK specimens (20 mm x 10 mm x 2 mm) were utilized. Specimens were split into 5 groups with 12 in each group, and they received the following treatments:

Group A: PEEK with biofilm without any treatment (Control). Group B: PEEK with biofilm that has been treated for 8 hours with 2% chlorhexidine. Group C: PEEK with biofilm and treated for 15minutes with 3.8% Sodium Perborate. Group D: PEEK with biofilm and 5 minutes of exposure to UV Rays at 254 nm. Group E: PEEK with biofilm and 10 minutes of exposure to UV Rays 254nm.

All of the PEEK specimens were then subjected to a three-point bending test on the Universal Testing Machine with a cross head speed of 5mm/second.

Result: The comparison of the mean CFU for candida albicans among all the 5 groups was Group A followed by Group D and Group B and negative values were seen with Group C and Group E. The comparison of the mean Flexural Strength (MPa) among all the 5 groups which showed a minimum value for Group E and maximum value for Group C.

Conclusion: Within the parameters of the investigation, it was possible to draw the following conclusions: 2% chlorhexidine was the most effective disinfectant, followed by 3.8% sodium perborate for 15 minutes, and UV radiation exposure for 5 minutes at 254 nm with almost identical efficiency and UV radiation of 10 minutes was the least effective. Overall, the flexural strength values were lowest after 10 minutes of 254 nm UV light exposure and maximum after 15 minutes of 3.8% sodium perborate.

Keywords: UV radiation, UV light, Flexural Strength.

Introduction

The necessity for dentures in older life was all but inevitable a century ago. Nowadays, 75% of persons over 65 still have some of their original teeth, although tooth loss, gum disease, dental decay, oral cancer, and mouth infections are still more common in older people. Aside from enhancing speaking and eating abilities, well-fitting dentures also increase self-confidence. It could also provide protection for the natural teeth that are still there and aid in preventing the face from ageing. The inability of elderly persons to remove oral plaque from denture appliances and teeth, however, leaves them more vulnerable to opportunistic oral mucosal infections, notably bacterial and fungal infections. Fungus-related infections have sharply grown in recent years, and are of utmost relevance due to the rise of immune impaired patients, such as cancer patients receiving chemotherapy and patients infected with the human immunodeficiency virus. In the latter category, it has a significant morbidity rate; over 85% of patients get an infection at some time during their illness. For those who wear dentures, denture stomatitis is a prevalent ailment. Due to its high virulence, capacity for adhesion, and capacity for biofilm development in oral tissues and denture surfaces, it is typically linked with Candida species, notably Candida albicans. A fungal infection of the oral mucosa known as Candida-Associated Denture Stomatitis (CADS) develops underneath the tissue surface of dentures. Its key characteristic is that antifungal medication alone cannot treat this ailment; also, predisposing factors must be eliminated or treated. According to research, 60–65% of people who wear dentures develop CADS. Numerous studies have studied the incidence of stomatitis brought on by dentures in men and women. According to some scientists, hormonal shifts may playa role in why women are more likely than males to develop denture-related stomatitis. In 1962, Newton divided denture stomatitis into three categories: punctiform hyperaemia, diffuse hyperaemia, and granular hyperaemia. The maxilla is where the alterations most frequently reveal themselves in partial and total denture wearers. CADS has a complicated and multiple etiology. The transition of Candida from the typical commensal flora (saprophytic stage) to a pathogenic form, which causes illness, may be caused by a number of local and systemic predisposing conditions. Respiratory tract illnesses may be brought on by denture plaque, which serves as a breeding ground for germs and fungus. Local factors like carbohydrate-rich diets, dry mouth, local trauma, complete dentures, unclean dentures, continuous denture wear, poorly fitting

dentures, and acidic salivary pH encourage the buildup of biofilm in the oral environment, encourage the development of Candida species, and alter the immune response of the oral mucosa. On the other hand, systemic factors like diabetes mellitus, immunosuppression (caused by things like chemotherapy, corticosteroids, immunosuppressive drugs, and biological therapies), immunodeficiencies (caused by things like HIV infection, acute leukaemia, and agranulocytosis), nutrition, and hematinic deficiencies (caused by things like iron, folate, and B12 deficiency), can affect how well the body defends itself. Controlling denture plaque biofilm is crucial for preventing oral and systemic diseases, and it also helps to regulate oral and systemic disorders in general.

The physical dexterity of denture users and their understanding of proper denture hygiene techniques may have an impact on biofilm management using conventional mechanical approaches like as brushing. Chemical cleaning techniques may be a great addition to mechanical ones since they lessen the number of bacteria that stick to dentures, make up for brushing's potential drawbacks, are simple to use, and are well-accepted by users. But these chemicals may be pricey, and if they're not handled correctly, they could ruin the foundation material for dentures or create discoloration. Material improvements can lead to technological and dental advancements. Modern materials utilized in advanced dentistry must have properties similar to dental structure, low plaque affinity, high aesthetics, and biocompatibility. It satisfies picky patients and aids in reconstructing tooth and dentition problems. The appealing contemporary substance PEEK (Polyether Ether Ether Ketone) is employed in Prosthodontics. It is utilized to make permanent and removably attached prostheses due of its advantageous chemical, mechanical, and physical qualities. PEEK is a semi-crystalline linear polycyclic aromatic polymer (-C6H4-OC6H4-O-C6H4-CO-). It was created by a team of English scientists in 1978 and later commercialized for use in industries. PEEK is a stiff polymer that is white, radiolucent, and has excellent thermal stability up to 335.8°C. Because of its unique chemical structure, which demonstrated stable chemical and physical qualities, studies have revealed that PEEK exhibits the least structural changes for steam, gamma, and ethylene oxide. PEEK, a versatile new dental material, may also be utilised to create the framework for permanent denture prosthetics. PEEK is a medical-grade polymer that offers patients an alternative to dentures made of chromium and cobalt. In view of the importance of proper denture cleanliness for oral and general health, this study intends to investigate the antifungal effectiveness of many commonly used cleaning agents. Therefore, a study is required to assess the impact of cleaning agents such as 2% chlorhexidine, 3.8% sodium perborate, and exposure to UV light for 5 and 10 minutes on the persistence of the Candida biofilm on PEEK, as well as to assess and compare changes in the material's mechanical properties following the cleaning protocols.

Methodology

PEEK specimen preparation: A total of 60 (20mm x 10mm x 2mm) PEEK specimens were abraded with grinding paper of grit 1200 (3M ESP sandpaper) and stored in water at room temperature for48 hours prior to disinfection using 70% alcohol/water solution for 10 minutes and air dried. (Fig.1and 2)Cultivation of C. albicans: Potato Dextrose broth was prepared based on manufacturer’s instructions. Standard strain of Candida albicans ATCC® 24433™ was grown and maintained in 10 ml of Potato Dextrose Broth by incubating at 24hrs at

27±2 °C. Following incubation, the culture was centrifuged at 8000 rpm for 10 minutes and washed with PBS (0.05M, pH 6.8). The supernatant liquid was discarded and the pellet containing microbial cells was resuspended to 1ml using broth. Cell density was adjusted to 1 x 104 CFU/mL using McFarland standards. (Fig.3a)

Contamination of PEEK specimens with C. abicans: All the PEEK Specimens were labelled and were placed into the 12 well plates containing 3ml of PDB broth with 1x104 CFU/ml of C. albicans. Plates were incubated at 27±20C with 5% CO2 for 24 hrs. (Fig. 3b)

Antimicrobial assessment of PEEK specimens: At the end of the incubation period, all the specimens were removed from wells, rinsed gently with PBS (0.05M, pH 6.8) three times to remove non-adherent cells. (Fig.4) Specimens were divided into 5 groups (n=12 per group) and subjected to treatment as following:

- Group A: PEEK with biofilm without any treatment – CONTROL GROUP (Fig.5)
- Group B: PEEK with biofilm - treated with 2% Chlorhexidine for 8 hours. (Fig.6)
- Group C: PEEK with biofilm - treated with 3.8% Sodium Perborate for 15 minutes. (Fig.7)
- Group D: PEEK with biofilm - exposed to UV Rays 254nm for 5 minutes. (Fig.8)
- Group E: PEEK with biofilm - exposed to UV Rays 254nm for 10 minutes. (Fig.9)

After disinfection, the treated specimens were rinsed/vortexed for 10 min in 1ml potatodextrosebroth taken each block individually as per the groups. The broth was serially diluted based on turbidity and 0.1 ml taken from each dilution was plated onto Potato dextrose agar plates and incubated for 24 hours at 27±2°C. Post incubation the surviving microorganisms were expressed as Colony Forming Units (CFU)/mL and the counts were averaged. Reduction in CFU was calculated by comparing microbial count in Control Group to surviving microbial count posttreatment in other groups. The effectiveness of each treatment was assessed based on the number of colonies and log reduction at end of each treatment groups when compared to control group.(Fig.10,11,12,13,14)

The evaluation of the PEEK specimens for flexural strength: Flexural Strength (MPa) was calculated using the Equation, MPa = 3PL/2bh2 wherein MPa is flexural strength, P is the load exerted on the surface, L is the span length, b is the specimen width, and h is the specimen thickness. (Fig.15)

$$\text{Flexural Strength, MPa} = \frac{3PL}{2bh^2}$$

After all the PEEK specimens were disinfected using various disinfecting protocols, the specimens underwent flexural strength testing using three-point-bending test performed by the Universal Testing Machine (BISS 858 Mini Bionix II, BISS Systems) in accordance to ISO 178, 02/1997 (Bending tests at synthetic materials) at a cross head speed of 5mm/second. The load exerted was recorded in Newtons (N). The specimens were placed upon two supports10 mm from each other and were exposed to the load of a wedge-shaped indenter with a curved edge of a cross section radius of 0.5mm. Loads were applied to the center of the samples using a UTM at a crosshead speed of 5 mm/min and a load cell capacity of 10 KN until breakage took place. The statistical evaluation of these findings was performed using the SPSS (Statistical Package For Social Sciences) version 20 (IBM SPASS statistics [IBM corp. released 2011]. The significance level for differences in the findings was at $p < 0.05$. The flexural strength is essential as it foresees the rigidness of the denture base

material that is vital to maintain the integrity of the soft as well as hard tissues along with the accurate fit. Hence, in this study we have evaluated and compared the flexural strength of PEEK denture material under various disinfecting materials to that of the Control Group.

Results

Table 1: Comparison of the mean CFU (log transformed data)among the groups using one-way ANOVA.

Groups	N	Minimum	Maximum	Mean	S.D	P value
Group A	12	2.36	2.50	2.420	0.044	0.001*
Group B	12	.11	.30	0.197	0.063	
Group C	12	-1.21	-1.03	-1.126	0.064	
Group D	12	-.19	.55	0.234	0.298	
Group E	12	-2.00	-1.11	-1.503	0.286	

Graph 1: Comparison of the mean CFU (log transformed) for C. albicans among all the 5 groups.

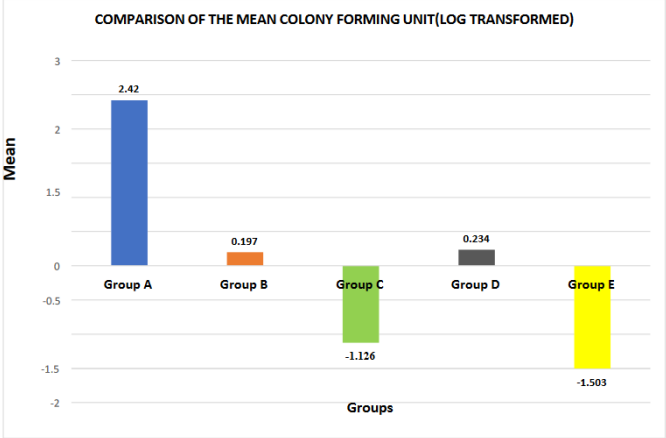
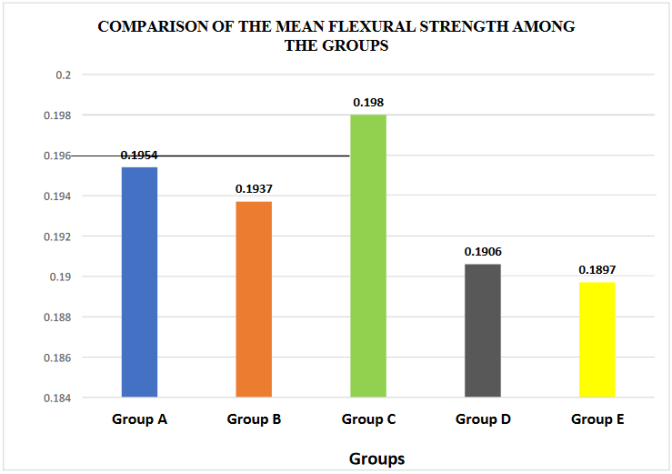


Table 2: Comparison of the mean flexural strength among the groups using one-way ANOVA

Groups	N	Minimum	Maximum	Mean	S.D	P value
Group A	12	.161	.215	.1954	.0152	0.589
Group B	12	.175	.215	.1937	.0113	
Group C	12	.172	.222	.1980	.0144	
Group D	12	.162	.211	.1906	.0149	
Group E	12	.168	.213	.1897	.0142	

Graph 2: The comparison of the mean flexural strength between the 5 groups



Discussion

A thin coating of pellicle, which is made up of numerous proteins, enzymes, and other molecules from saliva and covers newly introduced biomaterials into the oral environment, is instantly formed. However, saliva also includes antimicrobial proteins that prevent microbial growth and adhesion. On the one hand, saliva promotes microbial adherence. Contaminated prosthesis may result in cross-contamination between dental workers and patients. Additionally, denture plaque plays a significant role in the aetiology of opportunistic infections and respiratory tract infections brought on by aspiration in elderly individuals. An important step in preventing cross-contamination, enhancing patient health, extending the life of their dentures, and generally improving their quality of life is denture disinfection. For cleaning and maintaining the health of dentures, a variety of chemical and mechanical treatments are suggested. Chemical and mechanical methods are equally effective for halting and eradicating the formation of biofilm. Dentures are cleaned chemically by soaking them in antibacterial, antifungal, and solvent-containing liquids. These remedies may be utilised independently or in conjunction with mechanical or

ultrasonic cleansing. A disinfecting method should ideally be effective without affecting the properties of the materials used to create denture bases. Daily use of denture cleansers may have an influence on the physical and mechanical properties of the foundation material for dentures. When choosing a disinfectant fora dental prosthesis, it is crucial to consider compatibility with the type of material to be disinfected in order to avoid unfavourable effects.6,7 Colour, surface roughness, and hardness are the three characteristics that denture cleaners significantly impact, and which are essential for any prosthesis'long-term effectiveness. The rough surface of the denture base material is essential because microbe adherence to a surface is necessary for that surface to get colonised. The hardness of the denture base material affects both the ease of finishing off a material and its resistance to in-service scratches during cleaning operations.4,7 Dental materials that have changed colour indicate that they are either worn out or damaged. The colour stability of the denture base material may have a considerable impact on the materials' serviceability. In the realm of dentistry, PEEK is a potential biomaterial that might take the place of present polymers, metals, alloys, and ceramics. The innovative material PEEK is utilised to make both permanent and removably connected prostheses because of its superior chemical, mechanical, and physical properties. A strong, white, radiolucent polymer with exceptional temperature stability is PEEK. It is non-allergenic, has a flexural modulus of around 170-1000 MPa, and has a low plaque affinity. Human bone, enamel, and dentin have properties similar to those of Young's modulus and tensile strength. The substance is non-toxic, resistant to hydrolysis, and one of the most biocompatible substances available right now. It was determined that surface roughness had an impact on the microbiological adherence to PEEK denture base material. Additionally, it has been proposed that the wettability of a biomaterial may affect how a biofilm develops. Few studies have been done on how the surface chemistry of PEEK impacts the growth of biofilms. According to various articles, PEEK is an appropriate replacement for metals in prosthetic dentistry because it is less susceptible to microbial colonisation than other denture base materials. In dental treatment, which typically employs a wide range of alloplastic materials, PEEK is gradually replacing conventional dental materials. Additionally, to lessen the likelihood of problems like oral galvanism, many patients are requesting metal-free reconstructions. Due to its mechanical qualities, PEEK is a potential biomaterial that might replace existing polymers as well as metals, alloys, and ceramics in the area of dentistry. The current study aimed to evaluate and compare the effects of four different denture disinfectants on C. albicans efficacy as well as the modifications to PEEK denture base material's flexural strength after disinfection.

Chlorhexidine is one of the antibacterial substances that has garnered the most attention in recent years. It is recognised as the best antiseptic choice for avoiding dental caries, gingivitis, and stomatitis and is effective at reducing dental biofilm.3,4 Due to its capacity to cling to both soft and hard tissue and retain a potent prolonged release, it has emerged as the gold standard in dentistry.2,6In dentistry, sodium perborate monohydrate is used as a tool to help remove phlegm, mucus, or other fluids linked to an infrequent sore in the mouth, to clean small wounds, to temporarily treat canker sores, or to remove foreign objects from small wounds.6 Researchers Paranhos et al., Neppelenbroek et al., Ural et al., and Gornitsky et al.13 found that sodium perborate at a concentration of 3.8% is an effective denture cleaner

for preventing microbial colonisation and maintaining oral and denture health. They also found that the denture needs to be thoroughly cleaned with the 3.8% sodium perborate denture cleaning agent once a day for 15 minutes. Use of ultraviolet (UV) or visible (VIS) incoherent light is another interesting strategy that may be less hazardous. The findings of various investigations demonstrate that, due to its exceptionally low specific eradication coefficient, UV exposure for 5 minutes at 254 nm is by far the most effective wavelength for the eradication of Candida albicans followed by a longer exposure of 10 minutes. Since the results were inconsistent and there were substantial differences between the groups, a one-way ANOVA was performed to compare the mean CFU (log transformed data) across the sampled groups. In a comparison of the mean colony forming units (log transformed) for the five groups, Group A (Control Group) had the highest value at 2.24 CFU/mL, followed by Group D (UV exposure for 5 minutes) and Group B (2% chlorohexidine for 8 hours), while Group C (3.8% sodium perborate for 15 minutes) and Group E (UV exposure for 10 minutes) had the lowest values at -1.126 and -1.503 CFU/mL, respectively. In the current study, PEEK denture base material's flexural strength was assessed after being cleaned with several denture cleanser combinations. This study evaluated the mechanical characteristics of commercially available medical grade PEEK using three-point bending tests. using a universal testing machine (BISS 858 Mini Bionix II, BISS Systems) with a cross head speed of 5 mm/sec in line with ISO 178, 02/1997 ("Bending tests at synthetic materials"). A wedge-shaped indenter with a curved edge was used to indent the samples, which were supported by two supports spaced 10 mm apart and had a cross section radius of 0.5 mm. Loads were applied to the middle of the samples using a UTM at a crosshead speed of 5 mm/min and a load cell capacity of 10 KN until breaking took place. The amount of force used was expressed in Newtons (N). To calculate the flexural strength (Fs), an equation was utilised. The formula for flexural strength is Fs = 3PL/2bd2, where Fs stands for flexural strength, P for load, b for specimen breadth, L for span length, and d for specimen thickness. The mean flexural strength was compared across groups using the one-way ANOVA test, and between-group comparisons were made using the post-hoc Bonferroni test. One-way ANOVA was used to calculate the mean Flexural Strength (MPa) for all 5 groups after calculating the mean and standard deviation for each group. The p value from this study was (0.589).The mean flexural strength of the 12 PEEK specimens in each group was 0.1954 MPa with a standard deviation of 0.0152, while the mean flexural strength of the PEEK specimens in group B was 0.1937MPa with a standard deviation of 0.0113. The flexural strength of PEEK (Group C) was 0.1980 with a standard deviation of 0.0144, that of PEEK (Group D) was 0.1906 with a standard deviation of0.0149, and that of PEEK (Group E) was 0.1897 with a standard deviation of 0.0142. The find Ings revealed that Group C scored higher than Group A (the control) and Group B followed by Group Dand Group E. Study limitations,1. Since saliva was not employed in the current study's C. albicans culture, its influence cannot beexplained.2. It's also important to keep in mind that dental plaque biofilms are communities of several species, not just one, that interact intimately and respond to environmental changes as a single unit.3. However, given that repeated use of a disinfection method may produce alterations on the material's surface, hence enhancing the adherence of germs, additional study on

the influence of disinfection methods on the roughness of PEEK denture base materials is necessary.

Conclusion

Within the limits of the study, it could be concluded that:

- 0.197 CFU/mL of Candida albicans growth was seen when 2% Chlorhexidine was used as a disinfectant agent for 8 hours.
- -1.126 CFU/mL of Candida albicans growth was seen when 3.8% Sodium Perborate was used as a disinfectant agent for 15 minutes.
- The UV Radiation exposure of 5 minutes at 254nm showed 0.234 CFU/mL of Candida albicans growth.
- The UV Radiation exposure of 10 minutes at 254nm showed -1.503CFU/mL growth of Candida albicans.
- Overall, the most efficient disinfectant was 2% Chlorohexidine and almost similar efficacy was seen in UV Radiation exposure of 5 minutes at 254nm followed by 3.8% Sodium Perborate for 15 minutes and the least efficient was UV Radiation exposure of 10 minutes at 254nm.
- 2% Chlorohexidine exposed for 8 hours revealed a flexural strength of 0.1937 MPa, 3.8% Sodium Perborate exposed for 15 minutes revealed a flexural strength post disinfection with a value of 0.198 MPa, UV radiation exposure of 5 minutes at 254 nm revealed the flexural strength was effectively low at around 0.1906 MPa and 10 minutes of 254 nm UV radiation exposure revealed around 0.1897 MPa, the flexural strength was determined to be at its lowest. Overall, the flexural strength values were highest with 3.8% Sodium Perborate for 15 minutes and least with 10 minutes of 254 nm UV radiation exposure.

Fig.1: Each PEEK specimen of dimensions 20mm X 10mm X 2mm were milled using CAD-CAM.

Fig. 2: PEEK specimen after surface roughing to inoculate Candida albicans (right: rough surface, left: bottom)

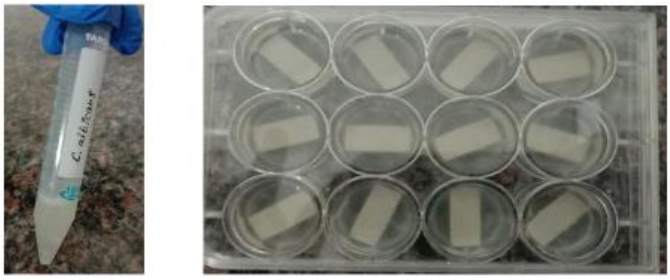


Fig. 3a: Candida albicans culture centrifuged and the pellet was resuspended to 1 ml potato dextrose broth (right); 3b: All the specimens were incubated with Candida albicans for 24hours with cell density adjusted to 1 x 104 CFU/mL (left).

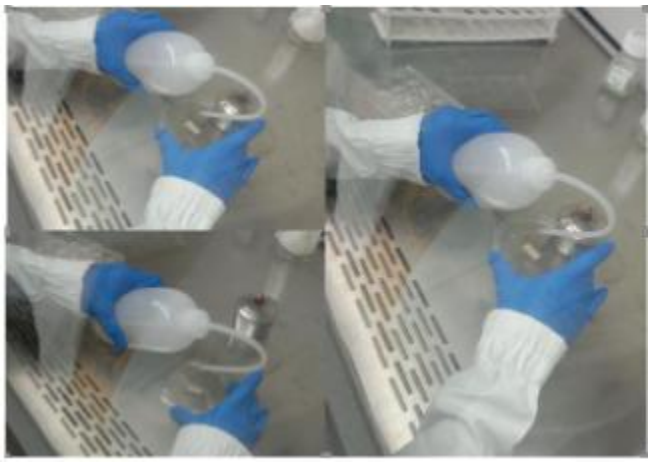


Fig. 4: After incubation with Candida albicans for 24 hours, all the specimens were removed from wells and rinsed gently with PBS 3 times to remove non- adherent cells.

Fig.5: After removing non-adherent cells the specimens were divided into 5 groups. Group A - Control group PEEK with biofilm without any treatment.

Fig.6: Group B - PEEK with biofilm with 2% Chlorohexidine for 8 hours

Fig.7: Group C - PEEK with biofilm with 3.8% Sodium Perborate for 15 minutes.

Fig.8: Group D - PEEK with biofilm with UV exposure for 5 minutes.

Fig. 9: Group E - PEEK with biofilm with UV exposure for 10 minutes

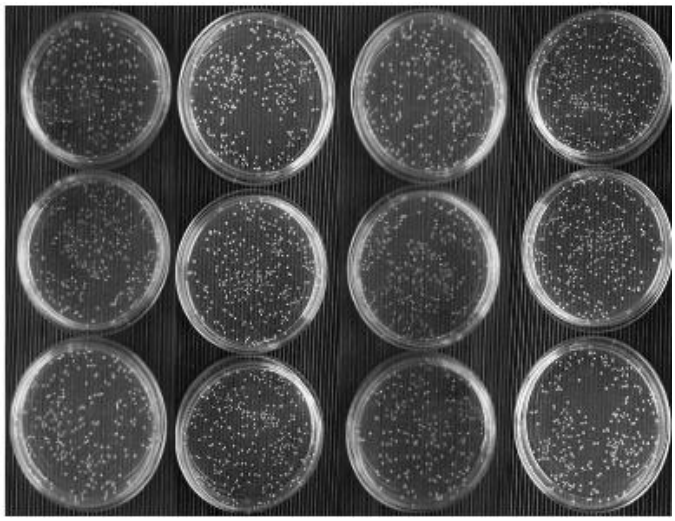


Fig.10: Group A – Candida albicans biofilm with no treatment.

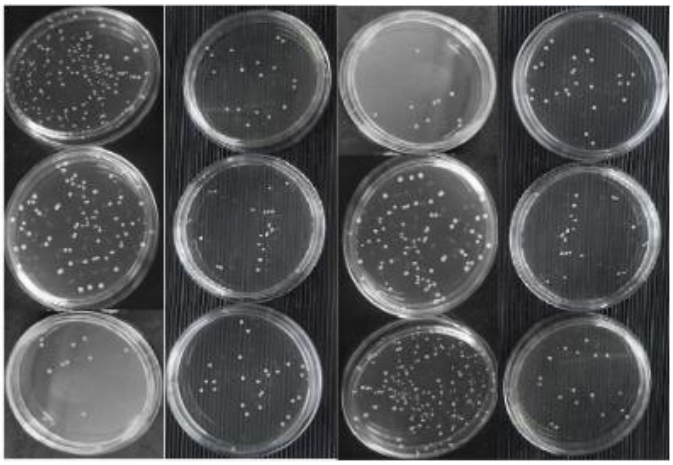


Fig.11: Group B – Candida albicans biofilm with 2% Chlorohexidine for 8 hours.

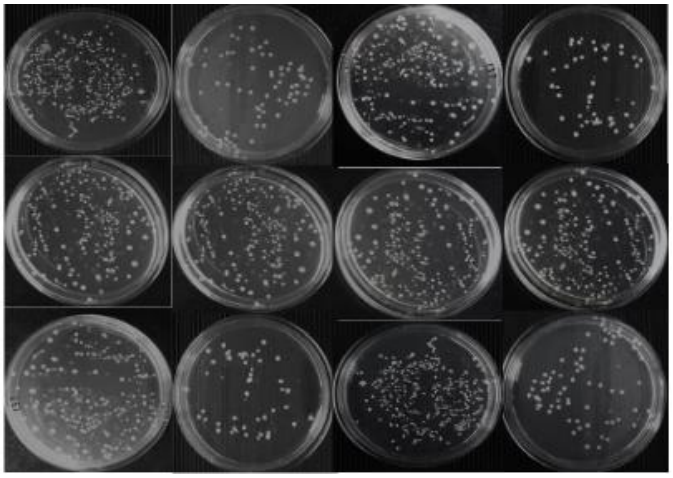


Fig.12: Group C - PEEK with biofilm with 3.8% Sodium Perborate for 15 minutes.

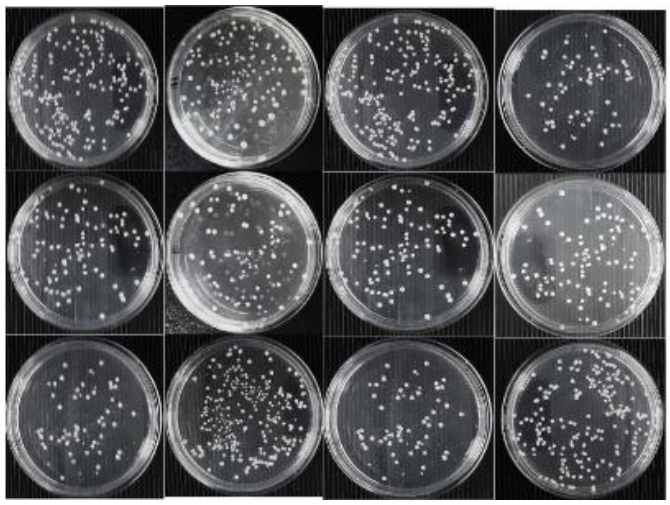


Fig.13: Group D - PEEK with biofilm with UV exposure for 5 minutes.

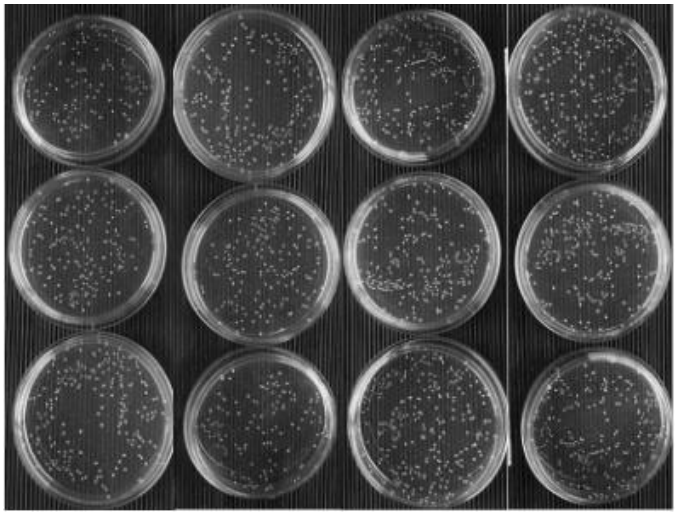


Fig.14: Group E - PEEK with biofilm with UV exposure for 10 minutes.

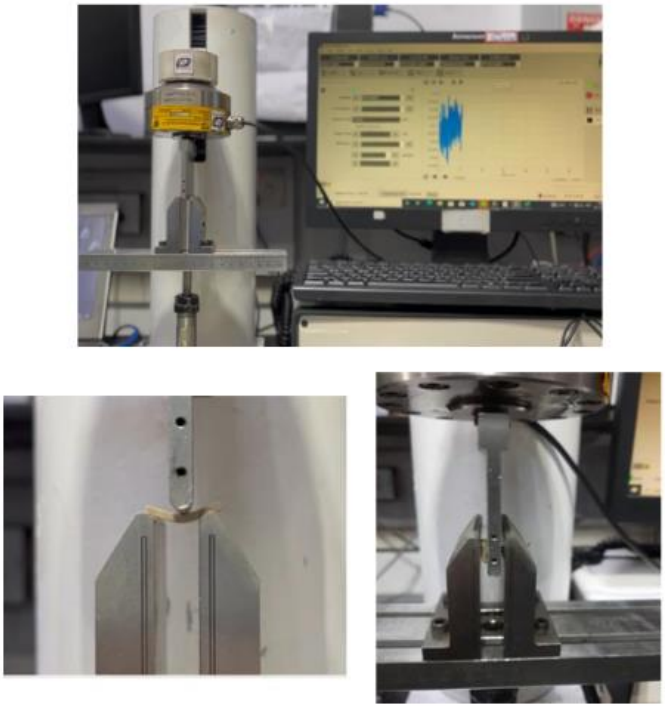


Fig.15: Flexural Strength Testing of PEEK specimens post disinfection using Universal Testing Machine (BISS 858 Mini Bionix II, BISS Systems)

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