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ORIGINAL RESEARCH

Radiofrequency Glow Discharge as a Mode of Disinfection for Elastomeric Impression Materials

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ABSTRACT

Introduction: Prosthodontic practice involves procedures in which impressions of the maxillary and mandibular arches are mandatory. Cross infection is one of the major problems that can occur in regular dental practice. Every dentist should take utmost care to prevent cross infection as oral cavity is the source of variety of microorganisms which can often cause diseases that can be fatal. Although precautions, such as wearing of gloves and mask, sterilization of instruments are given importance, the need for disinfection of impressions is often neglected. Hence, the aim of the study was to assess the disinfection potential of radiofrequency glow discharge (RGD) by microbiological studies.

Materials and methods: Disinfection potential of RGD on addition silicone (Reprosil, Dentsply, Milford DE, USA) was assessed. Total sample size was 20. Samples were divided into two groups of 10 each. Group I – control group and group II – RGD-treated group. Main groups were subdivided into subgroups A and B. Data collected were analyzed.

Results: The RGD-treated samples were found to be culture sterile which meant that there were no signs of growth of any organisms, thus proving the disinfection potential of RGD.

Conclusion: From this study, we can conclude that RGD is a very rapid and handy device, which can disinfect saliva contaminated elastomeric impression material surfaces.

Clinical significance: When compared with the difficulties and lack of efficiency encountered in disinfecting impressions by immersion and spray atomization, RGD can be very handy in dental clinics, as it is a very rapid and convenient method for infection control.

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INTRODUCTION

Dentists and supporting dental personnels are exposed to a variety of microorganisms which may lead to infectious diseases, such as acquired immunodeficiency syndrome, hepatitis B, tuberculosis, herpes I and II. Transmission of these diseases occurs due to contact with blood and other body fluids while performing dental procedures and handling contaminated instruments and impressions.[1,2] Hence, it is mandatory to follow infection control protocols in dental practice as advocated by American Dental Association (ADA) and other medical and dental councils.[3-6]

Disinfection is the destruction or removal of all pathogenic organisms or organisms capable of giving rise to infection. Oral cavity harbors a wide variety of microorganisms that can be infectious. Disinfection potential of various methods used depends on the technique, agent, concentration used, and exposure time.[7] Dental impression can give rise to transmission of microorganisms and infections as they are contaminated with blood and saliva during the impression procedures.[8] Disinfection provides a method of preventing the transmission of microorganisms from the patients to persons who handle the impression. Thus impression disinfecting methods have become a necessity.[9,10]

Various methods of disinfection of impression materials have been advocated. Immersion and spray disinfection are the most commonly used. Certain combinations of methods or disinfectants are more effective than others and their effect on the impression material can vary.[11,12]

Plasma treatment or radiofrequency glow discharge (RGD) has been suggested as one among the several methods of disinfection and has been proved to be effective in surface cleaning of implants.[13] Even though a number of methods for achieving disinfection are available, RGD proves to be a more rapid, recent, and scientific method for disinfection even though it is expensive.

Majority of restorative procedures are done by indirect methods. The basic requisites for the success of these indirect methods are an accurate impression and minute reproduction of the surface details, dimensional stability of the impression, and the ability to produce an accurate cast or die from the impression without loss of details. To meet these requisites, it is the best to use elastomeric impression materials.[14]

The ability to simultaneously disinfect the surface of a dental impression while enhancing the physical properties of the impression material suggests RGD approach as a rapid and convenient method of improving the production of prosthetic models and dies.[15]

The study was designed to assess the much predicted disinfection potential of RGD by various microbiological studies.

MATERIALS AND METHODS

The impression material used in the study was addition silicone (Dentsply, USA)

- Armamentarium for plasma treatment
  - RGD device

  The samples were subjected to plasma treatment in a radio frequency glow discharge unit (Edwards Vacuum Coating Unit, E 306 A, "Edwards" High Vacuum Ltd., Crawley, Sussex, UK). This unit consists of a cylindrical dome-shaped plasma reactor with ring form electrodes positioned at the height of 12 cm, from the base plate (Figs 1 and 2).

  The samples were placed at the center of the base plate in a Petridish. This position of the samples was standardized for the rest of the experiment.
- Armamentarium for sample preparation
  - Acrylic resin index that contained rectangular holes of dimension 20 × 10 × 2 mm
  - Glass slab
  - Glass plate
  - 99% ethanol
  - A load of 1 kg
- Armamentarium for microbiological studies
  - Glucose broth
  - Bile broth
  - Blood agar and MacConkey's agar plates.

Sample Preparation

A polyvinyl siloxane impression material (Reprosil, Dentsply, Milford DE, USA) was selected. Samples of the selected impression material were made using an ethanol-cleaned acrylic resin index that contained 20 × 10 × 21 mm rectangular holes. The acrylic resin index was placed on an ethanol-cleaned glass slab. The base paste and catalyst paste of polyvinyl siloxane impression material (Reprosil, Dentsply, Milford DE, USA) were mixed according to the manufacturer's instructions and placed into the holes of the acrylic resin index. It was covered with an ethanol-cleaned glass plate and was left to set for 10 minutes under a load of 1 kg. The samples were divided into two groups of 10 samples each. All samples were contaminated by keeping in the vestibular sulcus for 5 minutes.

Group I: Control group – 10 samples.

Group I was divided into two subgroups of five samples each.

- *Subgroup A*: Inoculated in glucose broth (1% glucose in nutrient broth)

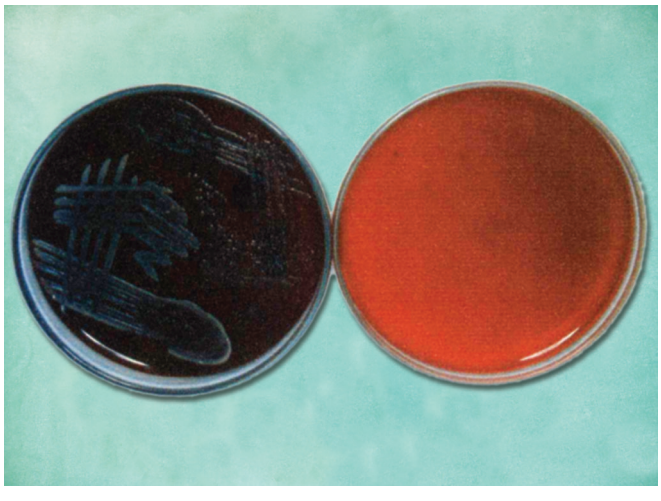

Fig. 1: Blood agar plate with multiple colonies and blood agar plate without colonies

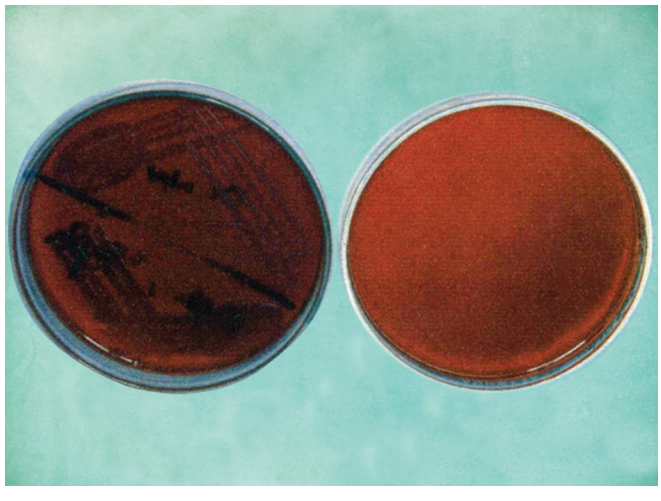

Fig. 2: MacConkey's agar plate with multiple colonies and MacConkey's agar plate without colonies

- *Subgroup B*: Inoculated in bile broth (0.5% bile salts in nutrient broth).

Subgroups A and B were incubated for 48 hours. Bacteria grow well in fluid media in 3 to 4 hours, so broth was used as enriched media before plating on solid media. The liquid media are not suitable for the isolation of organisms in pure culture and we cannot study the colony characters as well. Hence, culture was done to isolate the bacteria in pure culture by streak method of culture.

A platinum loop with 2½ inch wire and loop with 2 mm diameter was used. The loop was charged with the inoculum to be cultured (subgroups A and B separately) and was placed on the surface of dried plates of blood agar and MacConkey's agar in suitable dilutions toward the peripheral area. The inoculum was spread over different segments of the plate in a series of parallel lines. The plates were incubated at 37°C for 24 hours. Confluent growths of colonies were obtained over the final series of streaks. Colonies were identified using various biochemical tests.

The samples were subjected to RGD treatment for 30 seconds and then the samples were transferred immediately into the broths provided. In this case, also five samples were inoculated in glucose broth (subgroup A) and five samples in bile broth (subgroup B). The samples were incubated for 48 hours and then subculture was done on blood agar and MacConkey's agar plates and the plates were incubated at 37°C for 24 hours, and the results were analyzed.

Results for the Assessment of the Disinfection Potential of RGD

- Group I – subgroup A:
  - Culture characteristics:
    - On blood agar – small, white nonhemolytic, round, raised colonies
    - On MacConkey's agar – small, lactose fermenting, round, raised colonies.
  - Smear examination:
    - Smear examination showed Gram-positive cocci in clusters which may probably be *Staphylococcus*
  - Biochemical tests:
    - Catalase – positive
    - Coagulase test.

    Slide coagulase — Tube coagulase

    Negative — Negative
  - Colony identification:
    - The plates from subgroup A showed a heavy growth of coagulase-negative *Staphylococcus*.
- Group I – subgroup B
  - Culture characteristics:

    On blood agar:
    - Large, grayish-white, nonhemolytic, low convex colonies
    - Small, white, nonhemolytic, round, raised colonies.

    On MacConkey's agar:
    - Large, lactose fermenting, low convex, moist colonies
    - Small, lactose fermenting, round, raised colonies.
  - Smear examination:

    On smear examination
    - The colony was found to be Gram-negative bacilli
    - The colony was found to be Gram-positive cocci in clusters.
  - Biochemical tests:

    In the biochemical tests conducted, fermentation (lactose, sucrose, mannitol) tests were positive for group I and negative for group II. Catalase and coagulase tests were positive for both group I and II (Table 1).
  - Colony identification:

    The plates from the subgroup B showed a heavy mixed growth of *Escherichia coli* and *Staphylococcus aureus*. The growth of the following organisms was detected on the control specimens by various microbiologic studies.
    - Coagulase-negative *Staphylococcus*
    - *E. coli* and
    - *S. aureus.*
- Group II – RGD-treated group:

  The RGD-treated samples, which were inoculated in glucose broth and bile broth, were incubated for 48 hours and streak culture was done on blood agar and MacConkey's agar plate and was incubated at 37°C for 24 hours. It was found to be culture sterile which meant that there were no signs of growth of any organisms, thus proving the disinfection potential of RGD.

Table 1: Biochemical tests used

Biochemical tests	*(i)*	*(ii)*
Fermentation		
Lactose	+	–
Sucrose	+	–
Mannitol	+	–
Indole	+	–
Voges Proskauer	–	–
Urea hydrolysis	–	–
Nitrate	–	–
Oxidase	–	–
Catalase	+	+
Coagulase – Slide coagulase	–	+

DISCUSSION

Disinfection procedures must be performed correctly to assure effective microbial destruction with minimal damage to the impressions and maximum protection to the staff performing the procedures.[16] Rapid spread of hepatitis B virus, human immunodeficiency virus, and other microorganisms increased the need for prevention of cross infection in dental clinics and laboratories.[17] As per ADA guidelines methods of infection control should be incorporated in prosthodontic practice as impressions are major source for cross infection among dental specialists, dental laboratory technicians, and chair side assistants.[18]

The method of disinfection used for all materials are not the same as the degree of retention of viruses in each varies and the method used should not affect the inherent properties of the material and should provide complete disinfection. As the casts obtained from the impressions are an integral part in deciding the success of the prostheses, utmost care should be taken that the properties of impression materials and gypsum products are not affected by the method used. Casts obtained from impressions that are not disinfected will also be potential sources of infection.[19]

Among the various disinfection modes, immersion and spray disinfection of the impression are the most popular. Immersion disinfection is more reliable compared with spray disinfection although the latter appears easy and effective. Immersion disinfection ensures contact of the disinfectant on all the surfaces of the impression whereas in spray atomization, there will be pooling of the disinfectant in certain areas and complete disinfection cannot be ensured.[20]

Although disinfection of the stone casts by spray atomization has been advocated, it is not an ideal method to prevent cross contamination within the dental team.

The ADA has issued guidelines regarding the use of disinfecting agents for impression materials, which include the exposure time, dilution of the agent, and temperature at which it is treated.

Although the modes of disinfections of impression materials are numerous, there are various controversies regarding the effects of the disinfecting solutions on impression materials.[21] For example, Tullner et al[22] did not observe any negative effects after immersing polysulfide, polyether, and addition reaction silicone impressions in iodophor, sodium hypochlorite, and 5.25% glutaraldehyde. Results of studies conducted by Langenwalter et al[23] showed that same impression materials immersed in iodophor, sodium hypochlorite, glutaraldehyde, or twice deionized water or exposed to room air for 10 minutes did not produce any undesirable effects on the properties of these impression materials. Similarly, studies conducted by Matyas et al[24] and Bustos et al[25] concluded that immersion disinfection of elastomeric impression materials did not alter their inherent properties.

In a study conducted by Rios et al,[26] two polyethers and an addition silicone were disinfected using chlorine compounds, 2% neutral glutaraldehyde, and distilled water and no change in dimensional stability were detected. Minagi et al[27] observed that silicones showed only negligible change in dimensions on immersion disinfection.

Adverse effects of immersion disinfection of elastomeric impression materials have also been reported in studies conducted by Thouati et al.[28] Immersion in 5.2% sodium hypochlorite solution for 30 minutes caused expansion of the impressions, whereas no changes were seen in those immersed in quaternary ammonia and aldehyde solutions. According to Johnson et al,[29] for some elastomeric impression materials like polyether, immersion disinfection is contraindicated as they are sensitive to immersion in certain chemical agents. Dimensional changes have been reported in studies conducted by Owners and Goolan for polyether and condensation silicon when immersed for more than 5 hours. According to Johansen and Stacjouse, among the elastomeric impression materials disinfected by immersion disinfection in a glutaraldehyde solution for 16 hours, only addition silicone showed dimensional stability.

One of the most important factors to be considered in choosing the method for disinfection is the effect on the casts produced from the disinfected impression. In studies conducted by Peutzfeldt and Asmussen,[30] it has been reported that some disinfectant solutions cause detrimental effects on the cast surface due the impression/disinfectant combination. Utmost care should be taken while selecting the disinfectant solution for different impression materials and the exposure time also should be given importance.

The incompatibility of impression materials and the disinfectants may affect the accuracy, texture, dimensional stability, hardness, and other important aspects of the casts which are the deciding factors of an excellent prosthesis.[31] Moreover, the method used should not be messy and tedious as impressions are an integral part of our daily practice. Hence, it is essential to choose a method, i.e., ideal for the impression material to be disinfected. Novel methods of disinfection have been put forth among which disinfection by plasma treatment has gained acceptance.

Radiofrequency glow discharge or plasma treatment is a process in which plasma is generated through radiofrequency ionization of an inert gas. Energy is generated during the glow discharge/plasma cleaning procedure, which could clean the surface being exposed.[32,33] This has been proved to be useful in the sterilization of implants and various studies supporting the same have been

reported. Radiofrequency glow discharge is much handy and convenient procedure than immersion disinfection and spray atomization, which are the most commonly used modes of disinfection for impression materials.

The purpose of this study was to prove the disinfection potential of RGD. This was based on the reports from previous studies, which showed that plasma treatment method has been used effectively for surface cleaning of dental implants. Saliva contaminated samples of an elastomeric impression material were subjected to RGD exposure. The control groups of saliva contaminated samples were left untreated. The treated and untreated samples were inoculated in glucose broth and bile broth and incubated. As bacteria grow well in liquid media, broth was used as enriched media. However, liquid media are not suitable for isolation of bacteria in pure culture and study of colony characteristics. Hence, culture was done on solid media to isolate the bacteria in pure culture by streak method. A platinum loop with two and a half inch wire and loop with 2 mm diameter was used. The loop was charged with the inoculum to be cultured and was placed on the surface of dried plates of blood agar and MacConkey's agar in suitable dilutions toward the peripheral area. The inoculums were spread thinly over the plate in a series of parallel lines in different segments of plate and the plates were incubated at 37°C for 24 hours. Confluent growth of colonies was found at the site of primary inoculants. Well-separated colonies were found at the site of primary inoculant. Well-separated colonies were obtained over the final series of streaks. Various microbiologic studies were carried out and it was able to detect mainly three microorganisms namely, coagulase-negative *Staphylococcus*, *E. coli*, and *S. aureus* from the contaminated untreated specimens. The identification of bacteria was done by various biochemical tests after considering their culture characteristics on solid media. All the microorganisms detected were likely to cause opportunistic infections. However, in the case of RGD-treated specimens, not single microorganisms detected were likely to cause opportunistic infections. However, in the case of RGD-treated specimens, not a single microorganism was detected. The results of the study were in agreement with previous studies conducted on contaminated instruments. Thus, when compared with the difficulties and lack of efficiency encountered in disinfecting the impression by immersion and spray atomization, RGD device can be very handy in dental clinics, as it is a very rapid and convenient method for infection control.

From the study, it is evident that the high energy generated by the glow discharge provides complete disinfection of the impression material. The operating time is short and the equipment is relatively inexpensive. Considering the multiple applications of plasma treatment in dentistry, this device can be made commercially available with cost benefits. Further studies are indicated before this technique can be used clinically.

CONCLUSION

This study was conducted to prove the much predicted disinfection potential of RGD. For evaluating the disinfection potential of RGD, a polyvinylsiloxane impression material was selected and samples produced using an acrylic index. These samples were divided into two groups of 10 each. All samples were contaminated with saliva. The first group of 10 samples formed the control group. This group was again divided into two subgroups of five samples each. Subgroup A was inoculated in glucose broth and subgroup B in bile broth. Both were incubated for 48 hours. Later streak culture was done on MacConkey's agar and blood agar plates and incubated at 37°C for 24 hours. Well-separated colonies were obtained and they were identified using various biochemical tests.

Similarly, group II samples, which consisted of 10 samples, were subjected to RGD treatment and they were divided into two subgroups of five samples each. Subgroup A was inculcated in glucose broth and subgroup B in bile broth. These samples were incubated for 48 hours and subculture was done on blood agar and MacConkey's agar plates and incubated at 37°C for 24 hours and the results were analyzed. From this study, we can conclude that RGD is a very rapid and handy device, which can disinfect saliva contaminated impression material surfaces.

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