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Microwave Assisted Disinfection Method in Dentistry

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1. Introduction

Control of cross-infection has been a subject of interest to the dentistry area over the last few decades, due to the concern about the transmission of infectious-contagious diseases, such as AIDS, hepatitis, tuberculosis, pneumonia, and herpes, between the dental patients and dental personnel and the dental office and dental prosthesis laboratory (Codino & Marshall, 1976; Infection control in the dental office, Council on Dental Materials and Devices, 1978; Sande et al., 1975). This concern began in the 1970s, when the microorganism Mycobacterium tuberculosis was isolated in patients’ molds (Ray & Fuller, 1963) and it was found that Mycoplasma pneumoniae was transmitted to laboratory technicians when they performed wear on contaminated dentures (Sande et al., 1975). Several items, such as instruments (Tarantino et al., 1997), dental burs (Rizzo, 1993), impressions (Abdelaziz et al., 2004), gypsum casts (Berg et al., 2005; Davis et al., 1989), dental appliances (Rohrer & Bulard, 1985), and others are often heavily contaminated with microorganisms from saliva and blood. Powell et al. (1990) also observed that 67% of all the materials received in the laboratories, among them crowns, molds wax records, and dentures, were contaminated with pathogenic microorganisms. The constant exposure of dental personnel and instruments to saliva and blood in virtually every procedure is an ever present hazard and potential contributor to the transmission of infection. Therefore, the use of an inadequate disinfection procedure in the handling of dental materials not only places the unwary staff at risk, but also results in a high level of avoidable cross-contamination.

Dental practitioner has a legal and ethical responsibility to prevent infections in patients and staff members and an interest in protecting her-himself from contracting a disease from a patient. The Centers for Disease Control and Prevention of the United States (Kohn et al., 2004) have recommended sterilization and disinfection procedures to guarantee the safety of dental treatment in the dental health care settings. Typical methods of sterilization and disinfection include dry heat at 160 to 180°C for two hours, wet steam under pressure at 121°C for at least 15 minutes (autoclaving), immersion in chemical solutions, gamma radiation, and ethylene dioxide gas. However, these methods can be time-consuming and some of them require special and costly equipment, knowledgeable operative personnel, and constant surveillance. In order to overcome these limitations, microwave irradiation has
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been recommended as a practical physical sterilization method (Cottone et al., 1991) that is as effective as autoclaving (Tate et al., 1995). The lethal effects of a high frequency electric field on microorganisms was first described in 1925 (Kahler, 1929) and the destructive effects of strong radio-frequency fields on microorganisms were investigated in 1954 (Brown & Morrison, 1954). In fact, there are studies that have pointed out microwaves as a method for disinfecting food (Culkin & Fung, 1975), contact lenses (Hiti et al., 2001; Rohrer et al., 1986), laboratory microbiologic materials (Border & Rice-Spearman, 1999; Latimer, 1977), items of intimate clothing contaminated with Candida albicans (Friedrich & Phillips, 1988), hospital garbage (Hoffman & Hanley, 1994), coloring matter used in the cosmetic industry (Jasnow, 1975), and instruments used in medicine (Rosaspina et al., 1994). Hence, interest in this area has been maintained and in 1985 the technology was applied to the sterilization of dental appliances (Rohrer & Bulard, 1985).

In dentistry, microwave irradiation has been used for several purposes, including disinfection of toothbrushes (Nelson-Filho et al., 2011; Spolidorio et al., 2011), tongue scrapers (Spolidorio et al., 2011), instruments (Tarantino, 1997), contaminated gauze (Border & Rice-Spearman, 1999; Cardoso et al., 2007), dental burs (Fais et al., 2009; Rizzo, 1993), composite polishing instruments (Tate et al., 1995, 1996), molds made from elastomers (Abdelaziz et al., 2004), and gypsum casts (Berg et al., 2005; Davis et al., 1989). Furthermore, microwave irradiation has been widely accepted for polymerizing acrylic resin (Ilbay et al., 1994), drying gypsum products and investment materials (Hersek et al., 2002; Luebke & Chan, 1985; Luebke & Schneider, 1985; Tuncer et al., 1993), and as a post-polymerization treatment for reducing the residual monomer contents of polymerized acrylic resins and its cytotoxicity (Jorge et al., 2009; Nunes de Mello et al., 2003). Besides these purposes, some relevant applications of microwave energy in the dentistry field are the disinfection of removable dentures (Dovigo et al., 2009; D.G. Ribeiro et al., 2009; Rohrer & Bulard, 1985; Sanitá et al., 2009; Silva et al., 2006) and the use of this disinfection method to treat patients with oral candidiasis (Banting & Hill, 2001; Neppelenbroek et al., 2008; Webb et al., 2005). Although the lethal action of microwaves on microorganisms is well established in the literature, the mechanism of destruction of microwaves is not completely understood. While some studies sustain that the effect of microwave irradiation on microorganisms is directly of thermal character (Fitzpatrick et al., 1978; Jeng et al., 1987; Yeo et al., 1999), others claim that the killing of the organisms probably also results from the non-thermal effects of microwaves (Carrol & Lopez, 1969; Culkin & Fung, 1975; Olsen, 1965; Rohrer et al., 1986). In order to attempt these mechanisms of destruction of microwaves, many different microwave regimes have been tested and advocated (Banting & Hill, 2001; Neppelenbroek et al., 2008; Rohrer & Bulard, 2001; Sanitá et al., 2009). Efficacy of microwave irradiation seems to be associated with the vehicle in which the dentures are immersed, the time of exposure, the level of power of the microwave oven, and the type of microorganisms. In addition, when selecting a disinfection procedure, its effect on the physical and mechanical properties of the irradiated materials must be carefully considered. Thus, the establishment of different protocols must be essential to each particular case, with the goal of achieving consistent sterilization without harming dental materials.

Based on the given information, the purposes of this chapter are: 1. to explain and describe the range of applications of microwave irradiation in dentistry field; 2. to review the microbiological effectiveness and recommended protocols of microwave irradiation; 3. to show the mechanisms of action of the microwaves on the microorganisms; and 4. to discuss the effects of microwave irradiation on the properties of dental materials and appliances.
2. Applications in dentistry field

The recognition of the potential for transmission of numerous infectious microorganisms during dental procedures has led to an increased concern for infection control in dental practice. Approaches to the clinical use of microwaves for preventing cross-infection have shown relevant results. Devices and instruments used in dental offices have been identified as a source of cross-contamination among patients and from patients to dental personnel. With this in mind, investigations were undertaken to explore the efficacy of microwave irradiation in disinfecting dental mirrors (Tarantino et al., 1997) and handpieces (Rohrer & Bulard, 1985). In addition, dental burs, which may become heavily contaminated with necrotic tissues, saliva, blood, and potential pathogens during use, can also be sterilized by microwave irradiation (Fais et al., 2009; Rizzo, 1993; Rohrer & Bulard, 1985). In order to prevent cross-infection, microwave energy can also be used to the disinfection of finishing and polishing instruments (Tate et al., 1995, 1996). As any another device used in dental offices, finishing and polishing instruments routinely come into contact with patient’s saliva and blood and may also act as a source of cross-contamination. In accordance with the studies of Tate et al. (Tate et al., 1995, 1996), these dental devices can be effectively sterilized by microwave irradiation.

Another common dental procedure that may cause cross-infection, especially between patients and dental laboratory personnel, is the making of impressions. Previous studies showed that the majority of impressions arriving at a dental laboratory were contaminated with bacteria and other microorganisms (Egusa et al., 2008; Powel et al., 1990; Ray & Fuller, 1963; Sande et al., 1975; Sofou et al., 2002), irrespective of whether they had been exposed to a disinfectant procedure or merely rinsed with tap water (Sofou et al., 2002). The study of Egusa et al. (2008) showed an extensive contamination of alginate impressions with oral streptococci, staphylococci, *Candida* spp., methicillin resistant *Staphylococcus aureus* (MRSA), and *Pseudomonas aeruginosa*. These results indicate that a large number of microbes are retained on impression materials and are viably transferred onto the surface of stone casts. In fact, it has been shown that microorganisms can be transferred from impressions to gypsum models (Egusa et al., 2008; Leung & Schonfeld, 1983) and that the dental casts are potential sources of microbial transmission (Egusa et al., 2008; Leung & Schonfeld, 1983; Sofou et al., 2002). Also, even a cast from a properly disinfected impression may subsequently become contaminated by a technician or clinician. Considering all these information, microwave irradiation has been suggested to the disinfection of oral streptococci, staphylococci, *Candida* spp., methicillin resistant *Staphylococcus aureus* (MRSA), and *Pseudomonas aeruginosa*. These results indicate that a large number of microbes are retained on impression materials and are viably transferred onto the surface of stone casts. In fact, it has been shown that microorganisms can be transferred from impressions to gypsum models (Egusa et al., 2008; Leung & Schonfeld, 1983) and that the dental casts are potential sources of microbial transmission (Egusa et al., 2008; Leung & Schonfeld, 1983; Sofou et al., 2002). Also, even a cast from a properly disinfected impression may subsequently become contaminated by a technician or clinician. Considering all these information, microwave irradiation has been suggested to the disinfection of both impressions (Abdelaziz et al., 2004) and dental casts (Berg et al., 2005; Davis et al., 1989). The clinical relevance of impressions and dental casts microwave disinfection is that this procedure can be performed quickly and repeatedly, without the use of toxic, pungent, or allergenic chemicals. However, the disinfection of impression materials hinders possible cross-contamination only at the time the cast is poured. Because casts become contaminated after the intra-oral adjustments of dental appliances, they must be regarded as the major vehicle for cross-contamination and should be disinfected using microwave energy throughout all phases of the dental treatment.

Another application of microwave irradiation in preventing cross-infection is the disinfection of disposable materials. Although there is little scientific support for this purpose, some reports documented the disinfection of contaminated gauze and swabs (Border & Rice-Spearman, 1999; Cardoso et al., 2007) with the use of microwave irradiation. Cardoso et al. (2007) and Border & Rice-Spearman (1999) demonstrated that a short period
of exposure of contaminated gauze pieces and swabs to microwave energy (30 seconds) inhibit the growth of pathogenic microorganisms. The authors suggested that microwave oven could be used instead of an autoclave in a variety of clinical and research settings because the procedure is rapid and the equipment cost is minimal.

Among the purposes of microwave energy in cross-infection prevention, one of the most important is the disinfection of removable dentures. In addition to its contamination by the oral microorganisms, it has been reported that dentures are contaminated at various stages during their fabrication (Verran et al., 1996; Wakefield, 1980; Williams et al., 1985) and are capable of transmitting microorganisms to other materials, dental equipments, laboratory, and technicians (Kahn et al., 1982). Microscopic studies have also demonstrated that a biofilm similar to that formed on natural teeth is present on dentures (Budtz-Jorgensen & Theilade, 1983). Large quantity of Candida spp. (Budtz-Jorgensen & Theilade, 1983) and some bacterial species associated with systemic diseases have been found in removable dentures, with predominance of gram-positive bacteria, as Staphylococcus spp., Streptococcus spp., and Actinomyces spp. (Chau et al., 1995; Glass et al., 2001a; Monsenego, 2000). Gram-negative species, such as Neisseria perflava, P. aeruginosa, Klebsiella pneumoniae, and Enterobacter cloacae, have also been identified (Henderson et al., 1987; Latimer, 1977). In fact, in vivo studies (D.G. Ribeiro et al., 2009; Rossi et al., 1996) found C. albicans, S. aureus, Streptococcus mutans, and MRSA on the surfaces of dentures from patients, with C. albicans having the highest prevalence in these biofilms. Therefore, the denture can function as a reservoir of microorganisms, enabling the transmission of diseases in the dental office and from it to the prosthetic laboratories. Manipulation of dentures in the different dental procedures may also disseminate the microorganisms throughout the environment in the form of aerosols (Clifford & Burnett, 1995). These pathogens may be inhaled by the dentist, assistants, and laboratory technicians, resulting in cross-infection between patients and dental personnel. In the context of denture microwave disinfection, the first studies were performed in order to demonstrate the effectiveness of microwave irradiation in inactivating microorganisms adhered to complete dentures (Thomas & Webb, 1995; Rohrer & Bullard, 1985; Webb et al., 1998). Using several protocols, positive results were obtained by in vitro and in vivo studies, proving that microwave energy can be an effective method in the disinfection of dentures (Dovigo et al., 2009; Glass et al., 2001b; D.G. Ribeiro et al., 2009; Sanitá et al., 2009; Silva et al., 2006). Thus, performing microwave disinfection of a removable denture before it is transferred to a dental laboratory, and immediately before it is returned to the patient, provides a measure of infection control for all parties.

In the course of time, the fit of dentures progressively declines as a result of time-dependent changes in the supporting tissue. In this context, hard chairside reline acrylic resins or soft denture liners are proposed for permanent or temporary improvement of denture fit. These auto-polymerizing acrylic resins allow the clinician to reline a removable prosthesis directly in the mouth in intimate contact with a large area of oral mucosa. Although such liners improve the denture fit and offer comfort to some edentulous and partially edentulous patients, these materials may present a source of other problems. One of their greatest disadvantages is the ongoing task of hygiene maintenance. Denture reline materials, especially the soft liners, have been found to be more prone to microbial adhesion than acrylic resin base materials because of their surface texture and higher porosity (Nikawa et al., 1992). The rougher surface of a prosthesis with a soft liner exhibits greater colonization by C. albicans when compared with a conventional acrylic resin denture (Wright et al., 1985). As a result, the oral mucosa
is more susceptible to infections. A study evaluated the effectiveness of microwave disinfection on three hard chairside reline resins and observed a consistent sterilization after microwave exposure (Neppelenbroek et al., 2003). In other investigations (Dixon et al., 1999; Mima et al., 2008), microwave irradiation resulted in sterilization of a hard chairside reline resin and soft denture liners contaminated by four pathogenic microorganisms. It is also important to emphasize that disinfection by microwaves promotes inactivation of the pathogenic microorganisms present on both the surface and inside the pores of the acrylic materials (Chau et al., 1995; Dixon et al., 1999).

By being a reservoir of pathogens, the tissue surface of the acrylic resin denture enhances the infective potential of microorganisms and favors the appearance of oral infections (Budtz-Jorgensen, 1990). Oral candidiasis, represented by denture stomatitis in denture wearers, is one of the most common manifestations of disease associated with the use of removable dentures. Denture stomatitis is mainly caused by microorganisms of the Candida genus and normally affects the palate of approximately 65% of denture wearer patients (Chandra et al., 2001). This infection is characterized by the presence of multiple hyperemic points in the mucosa subjacent to the removable dentures of patients and, in more advanced cases, diffused erythematous areas and papillary hyperplasia of the palate may also be observed (Newton, 1962). Considering that microbial adherence and colonization on dental prostheses favor the appearance of denture stomatitis, the cleansing and disinfection of dentures is fundamental to prevent this disease. Recent studies have suggested denture disinfection by microwave irradiation for the treatment of denture stomatitis. The microwave regimes established in laboratory studies provided the baseline for subsequent clinical trials. Banting & Hill (2001) conducted the first study that evaluated the effectiveness of microwave energy for denture disinfection as a co-adjuvant in the treatment of denture stomatitis. The authors observed that the method was effective in the reduction of the clinical signs of infection. These findings are in agreement with those found by Webb et al. (2005) a few years later. A more recent study conducted by Neppelenbroek et al. (2008) also evaluated the effectiveness of complete denture disinfection by microwave energy in the treatment of patients with denture stomatitis. In agreement to Banting & Hill (2001) and Webb et al. (2005), it was observed that disinfection of the dentures by microwaves was effective for the treatment of denture stomatitis (Neppelenbroek et al., 2008). Investigations are still being carried out to evaluate the effectiveness of microwave irradiation in the treatment of denture stomatitis (Silva et al., 2008; Vergani et al., 2008). Further tests have been performed in order to evaluate the effectiveness of denture irradiation in the treatment of diabetic denture wearer patients with denture stomatitis, showing promising outcomes (Sanitá et al., 2010, 2011). Based on the above mentioned clinical studies, the use of microwaves has shown important results for the treatment of denture stomatitis. This disease is one of the most frequent opportunistic infections found in denture wearers, including the diabetic patients, and it may extend regionally and result in a systemic infection that is associated with high mortality rates (Colombo et al., 1999; Meunier-Carpentier et al., 1981). Hence, the prevention of colonization of the oropharynx is critically important in preventing systemic infections due to Candida.

A recent study has shown that pathogenic microorganisms can adhere in toothbrushes and tongue scrapers made from stainless steel- and polystyrene-based injection-moulded plastic (Spolidorio et al., 2011). Thus, toothbrushes and tongue scrapers become contaminated after use and, if not properly disinfected, may be a reservoir of microorganisms that maintain their viability for a significant amount of time, ranging 24 hours to 7 days (Nelson-Filho et
al., 2006). Microbial effectiveness of microwave irradiation has been documented in the literature and the effectiveness seems to be directly related to the protocol adopted. When defining a microwave irradiation protocol, the parameters to be considered are: the time of exposure; the level of power of the microwave oven; the material to be irradiated; the vehicle in which the material is immersed, and the type of microorganisms that colonize the material. Considering these parameters and the several applications of microwave energy in dentistry field, different protocols of microwave irradiation are available and have been tested.

There are some studies that evaluate microwave irradiation for the disinfection of dental air turbine handpieces (Rohrer & Bulard, 1985), mirrors (Tarantino et al., 1997), burs (Fais et al., 2009; Rizzo, 1993; Rohrer & Bulard, 1985), and finishing and polishing instruments (Tate et al., 1995, 1996). In the study of Rohrer & Bulard (1985), handpieces contaminated with a mixture of four aerobic bacteria (Staphylococcus epidermis, S. aureus, K. pneumonia, Bacillus subtilis, Clostridium histolyticum) and C. albicans were consistently sterilized with an exposure of 10 minutes at 720W to microwave irradiation when the materials were attached to a three-dimensional rotating device. These authors also observed that handpieces contaminated by both polio type 1 and herpes simplex type 1 viruses were consistently sterilized after an exposure to microwave irradiation. Another in vitro investigation proved that dental mirrors contaminated with S. aureus, B. subtilis, and Bacillus stearothermophilus can be sterilized by microwave irradiation at 600W for 4 minutes, with the instruments immersed in an aldehyde solution (Tarantino et al., 1997). A microwave regime of 10 minutes at 720W is sufficient to sterilize carbide and diamond burs contaminated with a mixture of S. aureus, K. pneumoniae, and B. subtilis (Rohrer & Bulard, 1985). In another effective protocol, carbide burs are individually placed in a loosely capped test tube with 10 mL of distilled water, transferred to the right lateral position inside the microwave oven, and then exposed to 5 minutes at 600W (Fais et al., 2009). A similar protocol consisting of 6 minutes of irradiation at 750W (Tate et al., 1995, 1996) can be adopted when disinfecting finishing and polishing instruments (Enhance finishing cups, L.D. Caulk Co., Milford, DE, USA and Min-Identoflex fine cups, Centrix Inc., Shelton, CT, USA).

Likewise, 10 minutes of irradiation at high power can sterilize customized impressions made from both vinyl polysiloxane (Cinch Platinum, Parkell, Farmingdale, NY, USA) and
polyether (Impregum F, 3M ESPE AG Dental Products, Seefeld, Germany) rubber impression materials (Abdelaziz et al., 2004). Microwave irradiation can also be used for gypsum casts disinfection (Berg et al., 2005; Davis et al., 1989). In the studies of Davis et al. (1989) and Berg et al. (2005), molds were contaminated with pathogenic microorganisms and the stone casts obtained were submitted to microwave irradiation. While *Serratia marcescens* cells on casts were inactivated by microwave irradiation at 900W for 1, 5 or 20 minutes (Davis et al., 1989), *S. aureus* and *P. aeruginosa* were killed after 5 minutes of irradiation (Berg et al., 2005). Microwave irradiation was ineffective in killing *B. subtilis* transferred to stone casts (Davis et al., 1989). Although a complete inactivation was not obtained, the gypsum casts submitted to microwaves for 20 minutes exhibited less growth than the samples irradiated for shorter times (Davis et al., 1989).

There are also two available protocols for the microwave disinfection of disposable materials, such as swabs and gauze. Cardoso et al. (2007) used stock cultures of *Escherichia coli*, *S. aureus*, *S. epidermidis*, *P. aeruginosa*, and *C. albicans* to contaminate gauze and swabs. Thereafter, the materials were placed into autoclave bags, sealed, and irradiated for 30 seconds at 1000W. This time/power regime was efficient in the sterilization of the disposable materials tested. When a lower power was used (650W), 30 seconds of microwave irradiation were also sufficient to sterilize gauze contaminated with the same pathogenic bacteria and fungi (Border & Rice-Spearman, 1999). With the regard of oral cleaning devices, disinfection of toothbrushes contaminated with the same pathogenic bacteria and fungi (Border & Rice-Spearman, 1999). 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obtained with the aforementioned microbiological studies, in which the test specimens or dentures were irradiated in a dry state, are variable with regard to the effectiveness of disinfection by microwaves. Since irradiation in water provides uniform heating of the materials, Dixon et al. (1999) suggested immersing the samples in water during exposure to microwaves. This procedure was considered adequate for eliminating microorganisms, including those located inside the pores. The authors inoculated *C. albicans* in resilient reliners and a heat-polymerized resin and showed that the specimens immersed in water during irradiation were completely disinfected after 5 minutes at maximum power, while those not immersed in water were only partially disinfected after the same time of exposure. Bearing in mind these results, Neppelenbroek et al. (2003) evaluated the effectiveness of a home microwave oven for the inactivation of *S. aureus*, *P. aeruginosa*, *B. subtilis*, and *C. albicans* present in three reliner resins. The contaminated samples were immersed in 200 mL of sterile distilled water and irradiated for 6 minutes at 650W. It was observed that all the test specimens were sterilized after irradiation, as no microbiological growth was noticed after the irradiated specimens had remained incubated for 7 days. Silva et al. (2006) evaluated the same protocol for disinfecting simulated complete dentures and observed that the lethal action varied according to the microorganisms tested. While complete disinfection was achieved for the dentures contaminated with *S. aureus* and *C. albicans*, those contaminated with *P. aeruginosa* and *B. subtilis* showed little, but detectable, microbial growth. The different results from those of Neppelenbroek et al. (2003) are probably related to the larger surface area of the complete dentures, given that the number of microorganism colonies on the acrylic resin surface is proportional to the total area involved. In addition, this difference could be related to the microorganisms tested. A greater resistance of *B. subtilis* to microwaves has been reported (Davis et al., 1989; Najdovski et al., 1991) and these results are probably related to the sporulation capacity of *B. subtilis*. Bacterial spores are metabolically inactive and particularly resistant to situations of stress, such as heating and radiation. Microwaves promote heating of the test specimens and water in which they are immersed, thus there is the possibility of spore formation during this procedure (Najdovski et al., 1991).

A more recent study (Mima et al., 2008) showed that the experimental protocols advocated by Dixon et al. (1999), Neppelenbroek et al. (2003), and Silva et al. (2006) could be used with lower exposure times. Test specimens contaminated with four microorganisms (*S. aureus*, *P. aeruginosa*, *B. subtilis*, and *C. albicans*) were immersed in water and submitted to microwave irradiation (650W) at different exposure times (5, 4, 3, 2, and 1 minutes). It was observed that all the test specimens irradiated for 3, 4, and 5 minutes were completely disinfected after microwave irradiation. When the time of irradiation was reduced to 2 minutes, the samples contaminated with *C. albicans* were completely disinfected while those inoculated with *S. aureus*, *P. aeruginosa*, and *B. subtilis* demonstrated microbial growth. When submitted to sterilization by humid heat, bacterial cells are inactivated at higher temperatures than fungal cells (Pelczar et al., 1993). Therefore, irradiation for at least 2 minutes promoted sufficient water heating to inactivate *C. albicans* but not the bacteria. Moreover, the yeast cells are larger than those of the bacteria (Verran & Maryan, 1997). Therefore, one could suppose that the *C. albicans* cells contained more water in their composition than did the other microorganisms tested, and therefore, they had been more susceptible to microwave irradiation. In spite of this in vitro study has evaluated small size test specimens that had surfaces with vitreous characteristics, differently from those observed clinically, its results indicated that the protocol should be evaluated in other experimental conditions. Therefore, laboratorial investigations were performed to evaluate the effectiveness of this protocol in...
the disinfection of dentures contaminated by several microorganisms. Sanitá et al. (2009) demonstrated that simulated complete dentures inoculated with different Candida spp. (C. albicans, Candida tropicalis, and the intrinsically resistant Candida glabrata, Candida dubliniensis, and Candida krusei) were completely disinfected by microwave irradiation for 3 minutes at 650W. Similar results have been reported for complete dentures contaminated with S. aureus and P. aeruginosa (Dovigo et al., 2009).

Given the promising results, some protocols were tested in clinical trials to evaluate the effectiveness of microwave irradiation in disinfecting patients’ dentures (Glass et al., 2001b; D.G. Ribeiro et al., 2009). In one study, fragments of dentures that had been worn for periods ranging from 12 days to 48 years were immersed in a chemical solution (MicroDent Sanitizing and Cleaning System®) and exposed to microwaves for 2 minutes (Glass et al., 2001b). This method showed positive results for denture decontamination, considering that no microbial growth was observed on the fragments. In addition, the study of D.G. Ribeiro et al. (2009) showed that 3 minutes of irradiation at 650W resulted in complete inactivation of the biofilm present on dentures of 30 patients. It emerged also from this study that a lower reduction in the count of microorganisms (C. albicans, S. aureus, and S. mutans) was observed when a lower time of exposure was used (2 minutes).

In terms of treating denture stomatitis, Banting & Hill (2001) conducted the first clinical study that evaluated the effectiveness of microwave energy for denture disinfection as a co-adjuvant treatment. This study was performed in 2001, when the effective protocol of 3 minutes at 650W had not yet established. Patients received topical antifungal medication (nystatin/three times a day) for 14 days and had their dentures irradiated (850W for 1 minute) on three different days (1st, 5th and 10th day). The authors observed that the method was effective in the reduction of the clinical signs of infection and of the invasive forms of C. albicans (pseudohyphas) adhered on the surfaces of the dentures. In another clinical study, microwave disinfection of dentures (10 minutes at 350W) in a daily basis during one week reduced the palatal inflammation and the numbers of Candida on cultures from the palates and dentures of patients (Webb et al., 2005). A more recent study (Neppelenbroek et al., 2008) also evaluated the effectiveness of denture microwave disinfection in the treatment of patients with denture stomatitis. The microwave treatment protocol adopted was 6 minutes of irradiation of the complete dentures at 650W, three times a week for 30 days. It was verified that disinfection of the dentures by microwaves was effective for the treatment of denture stomatitis and for the reduction of the mycelial forms of Candida spp. In agreement to Banting & Hill (2001), these authors also observed that the levels of recurrence of C. albicans on the internal surfaces of the dentures and the supporting mucosa were significantly reduced in the patients whose dentures were microwaved (Neppelenbroek et al., 2008). Other clinical investigations have been carried out to evaluate the effectiveness of microwave irradiation in the treatment of denture stomatitis. A modification on the protocol proposed by Neppelenbroek et al. (2008) was evaluated by Vergani et al. (2008) and Silva et al. (2008). These authors demonstrated that denture microwave irradiation for 3 minutes at 650W, three times weekly for 14 days, is able to reduce the clinical signs of denture stomatitis on the palatal mucosa and the Candida colonization on complete dentures. Further clinical evaluations have also been conducted in order to evaluate the effectiveness of this protocol in the treatment of diabetic denture wearer patients with denture stomatitis (Sanitá et al., 2010, 2011). It was observed that microwave disinfection of complete dentures, by itself, was as effective as nystatin, the more conventional topical antifungal medication, in reducing the Candida counts and the clinical signs of denture stomatitis infection in patients with diabetes mellitus.
Based on the aforementioned studies, it can be seen that several regimes of microwave irradiation in relation to time/power are available. The protocol must be selected in accordance to the specific application of the microwave energy. Regardless these parameters, microwave irradiation is a potentially effective method for inactivating various microbial species present on dental materials, many of which are related to oral pathologies.

4. Mechanisms of action on the microorganisms

While the inhibitory effect of microwave irradiation on microorganisms is being researched extensively, how microwave brings about this effect has been a matter of discussion. Some authors believe that microorganism inactivation by microwave irradiation is explained by a thermal effect (Fitzpatrick et al., 1978; Jeng et al., 1987; Yeo et al., 1999). Nevertheless, several studies suggest that, in addition to the heat generated around the microorganisms, there are other mechanisms resulting directly from the interaction of the electromagnetic field (Carrol & Lopez, 1969; Culkin & Fung, 1975; Olsen, 1965; Rohrer et al., 1986). Various mechanisms have been suggested to explain the nature of the so called non-thermal theory. Depending on the chemical composition of the microorganisms and the surrounding medium, the microbial cells may be selectively heated by the microwaves (Carrol & Lopez, 1969; Hiti et al., 2001; Yeo et al., 1999). Therefore, a certain frequency of microwave energy may be absorbed by certain fundamental biological molecules, such as the nucleic acids (Rohrer et al., 1986). Moreover, the level of molecular response from the biological system to the quantity of thermal energy may also explain the non-thermal effect of microwaves (Carrol & Lopez, 1969). The structural changes in the more peripheral layer around the biological macromolecules may alter their stability and function, and, consequently, these molecules may be denatured in an irreversible manner (Culkin & Fung, 1975). Studies have also demonstrated that the exposition of bacterial suspensions to microwave irradiation caused reduction on viable cell counts and increased the leaching of DNA and protein (Woo et al., 2000). These results suggest that microwaves caused changes in structural integrity and permeability of cell membrane and cell wall that have detrimentally affected the cell metabolism and lead to cell death (Campanha et al., 2007; Carrol & Lopez, 1969; Culkin & Fung, 1975; Olsen, 1965). Campanha et al. (2007) verified that leveduriform suspensions submitted to microwave irradiation at 650W for 6 minutes presented significantly lower cell count values and a larger number of substances released in comparison with the non-irradiated suspensions. The distinction between integral and non-integral cells was made based on the entry of methylene blue coloring into the cells, which is an indirect form of evaluating the cell membrane and wall integrity. Disintegrated cells were found in the irradiated suspension, indicating an alteration in the permeability or integrity of these structures. Moreover, the cells of this suspension lost their characteristic refringence, in spite of preserving their ellipsoidal leveduriform morphology. It was also demonstrated that after irradiation by microwaves, the release of electrolytes (K\(^+\), Ca\(^{++}\)) and nucleic acids was significantly higher in the irradiated suspensions than that from the non-irradiated (Campanha et al., 2007). However, despite of cell inactivation, the optic density and cell concentration were not altered in comparison with those of the control suspensions, indicating that cells were not completely lysed. Irrespective of the mechanism of microwaves on pathogenic microorganisms being thermal or non-thermal, it is known that the effect of inactivation occurs mainly in the presence of water, this being an important factor for sterilization in microwave ovens. Freeze-dried or dry organisms are unlikely to be
affected, even when submitted to prolonged exposures, indicating that humidity plays an important role in microwave energy absorption (Dixon et al., 1999; Watanabe et al., 2000). The water molecules present in the medium or inside the cells, being diploid, interact with the electromagnetic field of the microwaves. Consequently, numerous intermolecular collisions may occur and this vibration produces heating (Najdovski et al., 1991). This increase in temperature may cause protein and DNA denaturation (Ponne & Bartels, 1995). The consistent results from several studies, in which strains were completely inactivated when microwave irradiation was carried out with the specimens immersed in water, confirm this hypothesis.

According to scanning electron microscopic (SEM) studies (Neppelenbroek et al., 2003; Mima et al., 2008), microwave irradiation not only inactivated C. albicans, but also removed the nonviable yeast cells from resin surface. In this case, the irradiation was performed with the resin specimens immersed in water and, since water started to boil after approximately 1.5 minutes of irradiation, the movement of the water particles probably removed microbial cells from the resins. Verran & Marvan (1997) reported that the larger yeasts cells are more easily dislodged from acrylic resin surfaces compared with smaller bacteria. Considering the information discussed above, it can be concluded that, the nature of the lethality of the microwave irradiation for microorganisms may be a combination of thermal and non-thermal effects.

5. Effects on physical and mechanical properties of dental materials and appliances

Several investigations have focused on finding the adequate microwave parameters for microbial inactivation and cross-infection control. Different irradiation protocols have proved to be remarkably effective for disinfection of dental prostheses and other materials frequently used in dental practice. However, to enable this method of disinfection to be safely recommended, it is important to clearly demonstrate that it does not exert deleterious effects on the physical and mechanical properties of the materials submitted to microwaves. For this reason, studies have been conducted to examine the effect of microwave disinfection on dental instruments and burs, impressions, gypsum products, acrylic resins, denture lining materials, and artificial teeth.

Rohrer & Bulard (1985) exposed dental air turbine handpieces to microwaves for 2, 4, 6, 8, 10, and 15 minutes (720W). After 25 cycles of 10 minutes, the dental handpiece tested showed no decrease in the pressure reading and no apparent alteration in sound or cutting power. Another study (Tate et al., 1996) evaluated instrument performance of two composite finishing and polishing systems before and after three cycles of microwave irradiation (6 minutes at 750W). The sample surfaces were examined with a profilometer after the finishing procedure and the results demonstrated that the systems tested can be submitted to microwave irradiation at least three times without affecting performance. Questions have also been raised about the effects of microwave regimens on the microscopic characteristics, durability, and strength of dental burs, which can have their sharpness and ability to effectively cut tooth structure altered. The effect of sterilization with microwaves on diamond burs was evaluated by Rizzo (1993). The author evaluated the dental burs by viewing them under stereomicroscope before and after sterilization cycles. It was found that no damage was present after 15 cycles. The possible influence of microwave irradiation on the cutting capacity of carbide burs was also investigated (Fais et al., 2009). The burs were
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used to cut glass plates in a cutting machine set for 12 cycles of 2.5 minutes each and, after each cycle, they were exposed to microwave irradiation for 5 minutes at 600W. The cutting capacity of the burs was determined by a weight-loss method. Compared to the control conditions, the microwaved burs showed a statistically significant decrease in their cutting capacity. Thus, microwave irradiation requires further investigations before final recommendations can be made for disinfection of carbide burs.

Microwave disinfection of rubber impressions was also suggested by some authors as an alternative approach of controlling microbial transmission. In this context, the reproducibility of rubber impressions after microwave irradiation (10 minutes/720W) has been evaluated and the results compared with other disinfection methods (Abdelaziz et al., 2004). Microwave sterilization had a small effect on accuracy of impressions and this procedure has been recommended as a suitable technique for sterilizing rubber impressions. Another application of microwaves in dentistry is the disinfection of gypsum casts. Although there are no studies evaluating the disinfection protocols on the properties of gypsum materials, the effect of drying casts by microwaves has been described. In this context, microwave irradiation of gypsum casts has been tested as to its effect on the resistance to fracture (Hersek et al., 2002; Luebke & Schneider, 1985; Tuncer et al., 1993) and hardness (Luebke & Chan, 1985). In general, the results indicated an improvement in these properties. However, there was some concern that a decrease in the compressive strength and the appearance of cracks or porosities in the surface might occur when gypsum casts were exposed to irradiation with a very high power (1450W). Other physical and mechanical properties, such as abrasion resistance and dimensional stability, should be performed to confirm the clinical applicability of this procedure.

One of the main applications of microwaves in dentistry is to disinfect dental prosthesis. A large number of investigations have been published in the past and recent years concerning its effectiveness and limitations. Laboratorial investigations aimed at identifying if microwave exposure affects the surface hardness of the denture base acrylic resins, relining materials, and artificial denture teeth. The hardness of a material is the result of the interaction of several properties, such as ductility, malleability, and resistance to cutting, and, therefore, hardness tests may be used as an indicator of these properties (Anusavice, 1996). Also, the hardness of materials is related to its resistance to local plastic deformation. Two universal types of microhardness test, Vickers and Knoop, are standard methods for measuring hard surfaces, while the Shore A measures hardness in terms of the elasticity of the material. It has been demonstrated that the Vickers hardness of a heat-polymerizable acrylic resin was not changed after different times of exposure to microwaves (6, 5, 4, 3, 2, and 1 minutes) at 650W (Machado et al., 2009; D.G. Ribeiro et al., 2008). The Knoop hardness values of a denture base resin were also not changed after two cycles of microwave disinfection for 6 minutes at 690W (Sartori et al., 2008). In addition, no alterations in the Shore A hardness values were detected after microwave irradiation (3 minutes/500W) of resilient relining materials (Pavan et al., 2007). Other studies showed, however, increased hardness of denture base materials associated with microwave irradiation. Polyzois et al. (1995) evaluated two microwave disinfection protocols on the hardness of test specimens of a heat-polymerizable resin (3 or 15 minutes at 500W). Both protocols provided an increase in microhardness values. Similar findings were described by Machado et al. (2005) for two resilient relining materials submitted to seven irradiation cycles for 6 minutes (650W). D.G. Ribeiro et al. (2008) evaluated the effect of different times of exposure to microwaves (5, 4, 3, 2, and 1 minutes/650W) on the hardness of four relin materials and the findings suggested
that the disinfectant method promoted an increase in the hardness of the relining resins. The increase in microhardness values after microwave disinfection may be related to the increase in temperature during the irradiation procedure. Arab et al. (1989) reported an increase in hardness values when heat-polymerizable resins were immersed in water heated to 100ºC. Similarly, an increase in Vickers hardness of a relining resin after heat treatment in a water bath at 55ºC for 10 minutes has been reported (Seó et al., 2007a). These results may be attributed to the reduction in the level of residual monomer, as a result of the complementary processes of polymerization (Lamb et al., 1983; Sideridou et al., 2004) and diffusion of residual monomer, both favored by the increase in temperature during microwave irradiation.

Besides the acrylic denture base resins, dentures also comprise artificial teeth. For this reason, the effect of microwave disinfection on the surface hardness of artificial teeth commonly used for denture construction was also evaluated (Campanha et al., 2005). Two microwave disinfection cycles of 6 minutes each (650W), preceded or not by immersion in distilled water for 90 days, were tested. From these experiments, a reduction in surface hardness of the artificial denture teeth was observed after microwave irradiation. It seems that the high temperature associated with the movements of the molecules probably caused an increase in the speed of diffusion of the water molecules into the polymer, facilitating the movement of the polymeric chains during performance of the hardness (Takahashi et al., 1998). Thus, it is feasible that the reduction in hardness after irradiation is related to water sorption rather than to microwave irradiation. In fact, the teeth from the group that was microwaved after 90 days of water saturation presented no significant alteration in hardness after disinfection.

Since microwave disinfection involves the exposition of dentures to water at high temperature, it has been hypothesized that this may affect the bond strength between the artificial teeth and the acrylic resin from which dentures are made. Results from a study evaluating the effect of microwave disinfection (6 minutes/650W) on the bond strength of two types of denture teeth to three acrylic resins showed that microwave disinfection did not adversely affect the bond strength of all tested materials, with the exception of one tooth/resin combination (R.C. Ribeiro et al., 2008). In another study microwave irradiation for 3 minutes at 650W promoted a reduction in the impact strength of the tooth/acrylic resin interface, which could be explained by the increase in the degree of conversion of self-polymerizing resin, reducing the cohesion at the interface of the samples (Consani et al., 2008a).

The effects of microwave irradiation on other surface properties, such as roughness and porosity, have also been investigated. According to Allison & Douglas (1973), smoother surface retain a smaller quantity of biofilm, thus avoiding the proliferation of microorganisms on the acrylic surface of dentures. Surface roughness is an important characteristic of dental materials and, therefore, there is a direct correlation between the values of roughness and bacterial adherence. Moreover, according to Yannikakis et al. (2002), the presence of pores may reduce the mechanical properties of acrylic resin, as well as interfere in denture hygiene. It seems that the effect of microwave irradiation on a denture’s surface roughness and porosity depends on the time of exposure, number of cycles, and the type of denture resin used. Novais et al. (2009) investigated the occurrence of porosity on the surface of four self-polymerizable acrylic resins and one heat-polymerizable resin, after two or seven cycles of microwave disinfection (6 minutes/650W). The number of pores found in two out of five resins remained similar after microwaving, while a reduction in porosity was observed in two resins after seven disinfection cycles. In these cases, it was...
suggested that the high temperatures may have been attained during exposure to microwaves, which led to a greater degree of conversion and continuation of polymerization. Seven cycles of microwave disinfection increased the number of pores in one material. According to the authors, this material presented a high level of residual monomer and, therefore, a probable explanation for the increase in the number of pores in this resin was related to monomer vaporization. Another investigation also showed that the use of microwave energy can modify the surface texture of acrylic resins (Sartori et al., 2006).

It was reported that two microwave disinfection cycles (6 minutes at 690W) promoted an increase in surface roughness of an acrylic resin. However, only one material was evaluated and the effect of reduced exposure times on surface roughness was not investigated.

From the literature, it seems that microwave disinfection can play a role in promoting changes in denture materials. The flexural strength of five chairside reline resins and one denture base resin were evaluated after two and seven cycles of microwave disinfection at 650W for 6 minutes (Pavarina et al., 2005). The flexure strength of three resins presented significant increase in strength values. In contrast, two reline resins presented reduced flexure values after microwave irradiation. The heating provided by each of the seven cycles of microwave disinfection may have increased the absorption of water of some of the evaluated materials, resulting in a reduction in the flexural strength values. In view of these results, shorter times of exposure to microwaves, and their effects on the flexural strength of acrylic resins, were investigated and the findings showed that the flexural strength of four reline materials and one heat-polymerizable resin was not detrimentally affected after 5, 4, 3, 2, and 1 minutes of microwave irradiation at 650W (D.G. Ribeiro et al., 2008). Indeed, the disinfection method was capable of significantly increase the flexural strength of one reline resin. A similar result was described elsewhere after a disinfection cycle for 3 minutes at 650W (Consani et al., 2008b). In addition, another investigation (Polizois et al., 1995) observed that the flexural properties of a heat-polymerizable resin remained unaltered after the use of a low power (500W) associated with a long exposure time (15 minutes). However, in this study the samples were irradiated in a dry condition, a procedure that has been shown to be less effective for microbiological inactivation. In a more clinically relevant approach, the effect of denture microwave disinfection on the maximum fracture load, deflection at fracture, and fracture energy of intact and relined denture bases was evaluated. After exposed to microwave irradiation for 7 days (6 minutes/650W), the strength of the denture bases was similar to the strength of those immersed in water for 7 days (Seó et al., 2008).

An aspect that has also been investigated is the influence of microwave disinfection on bond strength between resilient liners and denture base acrylic resin. Test specimens made of resilient resins bonded to a denture base resin were submitted to microwave disinfection for two and seven irradiation cycles of 6 minutes at 650W (Machado et al., 2005). Microwave disinfection did not compromise the adhesion of resilient liners to the denture base resin. In a subsequent study, seven microwave disinfection cycles (6 minutes/650W) did not decrease the torsional bond strength between two hard reline resins and a denture base resin (Machado et al., 2006). Therefore, in general, the use of microwaves for denture disinfection does not appear to have any negative effect on the bond strength of reline materials frequently used in dental practice. Recently, a clinical study (R.C. Ribeiro et al., 2009) also reported the color stability of a hard chairside reline resin after microwave disinfection. Another important aspect that should be considered prior to the selection of a disinfection procedure is the maintenance of adequate adaptation between the denture base and residual
ridge. Clinically, this characteristic is fundamental both for denture retention and the preservation of the supporting tissues. Changes in denture base adaptation could act as one of the causes of alveolar bone loss and be indirectly responsible for decreasing the retention and stability of the denture (De Gee et al., 1979). A denture that exactly reproduces the supporting tissue may assure a uniform distribution of forces on the largest possible area of surface. Thus, analyses of the effect of microwave disinfection on the dimensional stability of denture bases resins have been conducted. Burns et al. (1990) aimed to determine the possible influence of microwaving on the dimensional stability of heat-polymerizable, self-polymerizable, and light-polymerizable resins. Test specimens were fabricated and submitted to measurements of mass and length before and after microwave irradiation (15 minutes/650W). The results showed that all the materials maintained dimensional stability after the disinfection procedure. In another study, microwave disinfection (3 or 5 minutes at 650W) promoted small, clinically insignificant dimensional changes on test specimens of a heat-polymerizable resin (Polyzois et al., 1995). Contrasting results were obtained in the study of Gonçalves et al. (2006), who evaluated the effect of two or seven cycles of microwave disinfection (6 minutes/650W) on the linear dimensional change of four reline resins and one denture base resin. Three of the evaluated resins presented significant alteration in the linear dimensional after disinfection. In spite of the positive and negative findings found by these different studies, all of the presented results cannot be extrapolated directly to a clinical situation, since the test specimens used did not simulate the dimensions and shape of denture bases. Standardized dentures were fabricated in the study of Thomas & Webb (1995) in order to evaluate the effect of microwaves on their dimensional stability. After 10 minutes of exposure to microwaves at 604W, some of the areas measured in the dentures presented significant shrinkage or expansion. Similarly, Sartori et al. (2008) observed that the disinfection cycles of 6 minutes (690W) could compromise the dimensional stability of denture base resins. In another study, however, a lower power setting (331W) decreased the occurrence of dimensional changes when the dentures were microwaved for 6 minutes (Thomas & Webb, 1995). The protocol of 6 minutes/650W of microwave irradiation was also tested for the dimensional stability of denture bases (Seo et al., 2007b). Repeated disinfection cycles were performed (two and seven) and an increase in shrinkage was observed both in intact bases and bases relined with heat-polymerizable resins. As the bases were immersed in water during irradiation, the results could be related to the possible occurrence of complementary polymerization as a result of water heating. In another investigation, the use of the same microwave power (650W), but with irradiation for 3 minutes, promoted no deleterious effects on the adaptation of denture bases (Consani et al., 2007). In fact, the authors observed that microwave irradiation improved the adaptation of bases in some experimental conditions. Pavan et al. (2005) also suggested that use of shorter irradiation times preserves the dimensional stability of dentures. The authors fabricated 30 denture bases that were submitted to irradiation for 3 minutes/500W or 10 minutes/604W. No dimensional alteration was observed in the bases irradiated for 3 minutes. Taken together, the results from all the cited studies suggest that short microwave irradiation times should be used, so that the adaptation of denture bases to subjacent tissue is not changed. Recently, Basso et al. (2010) performed a clinical evaluation of the effect of 3 minutes of microwave irradiation at 650W once or three times a week on the linear dimensional stability of complete dentures. Measurements were taken before the first microwave disinfection (baseline) and after each week of disinfection. Furthermore, the dentures were monitored clinically. Three times weekly irradiation showed significantly
higher shrinkage in all evaluated weeks. This result could be attributed to the heating generated by microwave irradiation in an already polymerized material. Even though three microwave disinfections showed statistically significant greater shrinkage, the clinical evaluation did not reveal any change. Therefore, the authors suggested that microwave irradiation can be used clinically for the disinfection of dentures and treatment of denture stomatitis.

Given the information above, it is clear that discordant results have been published regarding the risks of denture microwave disinfection. It seems that the occurrence of negative effects on physical and mechanical properties of dentures depends on the microwave protocol tested and type of material evaluated. It also seems that the use of short exposure times could minimize the occurrence of harmful effects on the dental prostheses. Taking all the results into account it would seem that the microwave regime of 3 minutes at 650W is adequate for denture disinfection without causing significant detrimental effects on the denture materials.

6. Conclusion

According to the information discussed in this chapter, there is scientific evidence to support the efficacy of microwave irradiation in preventing cross-infection and treating denture stomatitis. Several protocols of irradiation were described and discussed, and the microbiology effectiveness of microwave energy was clearly demonstrated. Regardless of all the parameters used, we can concluded that microwave disinfection is an effective, quick, easy, and inexpensive versatile tool that can be performed by dentists, assistants, technicians, patients and/or their caregivers to inactivate microorganisms. In addition, the use of a microwave oven does not require special storage and does not induce resistance for fungi or other microorganisms. Thus this method may have an important potential use in dental offices, dental laboratories, and institutions and hospitals in which patients are treated, especially those wearing removable dentures.

7. References


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The Microwave heating has not only revolutionized the food industry but also has extended its wings widely towards its multidimensional applications. Thus it has opened new vistas of potential research in science and technology. The book is compiled into Seventeen Chapters highlighting different aspects varying from epistemological discussion to applicability of conceptual constructs. The inclusion of discussion on the avenues in the field of Chemistry, Health & Environment, Medical Sciences and Technology makes it an exquisite work for the aspirant Researchers. As the text book for the beginners, it is designed fundamentally to be a reference monograph to the experts providing a passage for future research. The plethora of literatures are available on Microwave Applications but they seldom direct their readers to concentrate on the key aspects behind the success in microwave applications in different fields. Here is the attempt to fill up the gap with this book.

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