Introduction

The major causative role of micro-organisms in the pathogenesis of pulp and periapical diseases has clearly been demonstrated. The main aim of endodontic therapy is to disinfect the entire root canal system, which requires the elimination of micro-organisms and microbial components and the prevention of its reinfection during and after treatment. This goal is pursued through chemomechanical debridement, for which mechanical systems are used with irrigating solutions.

Standard endodontic irrigation protocol

Sodium hypochlorite

Sodium hypochlorite (NaOCl) is the main endodontic irrigant used, owing to its antibacterial properties and its ability to dissolve organic tissue. Sodium hypochlorite is used during the instrumentation phase to increase its time of action within the canal as much as possible without it being chemically altered by the presence of other substances. The effectiveness of this irrigant has been shown to depend on its concentration, temperature, pH solution and storage conditions. Heated solutions (45–60 °C) and higher concentrations (5–6 %) have greater tissue-dissolving properties. However, the greater the concentration, the more severe the potential reaction if some of the irrigant is inadvertently forced into the periapical tissue. In order to reduce this risk, the use of specially designed endodontic needles and an injection technique without pressure is recommended.

EDTA

The main disadvantage of NaOCl is its inability to remove the smear layer. For this reason, combination of NaOCl with EDTA (ethylenediaminetetraacetic) is recommended. EDTA has the ability to decompose the inorganic component of intracanal debris and is generally used in a percentage equal to 17 %. EDTA appears to reduce the antibacterial and solvent activity of NaOCl; thus, these two liquids should not be present in the canal at the same time. For this reason, during mechanical preparation, abundant and frequent rinsing with NaOCl is performed, while the EDTA is used for 2 min at the end of the preparation phase to remove the inorganic debris and the smear layer from the canal walls completely.

Ultrasonic activation of NaOCl

The use of ultrasound during and at the end of the root canal preparation phase is an indispensable step in improving endodontic disinfection. The range of frequencies used in the ultrasonic unit is between 25 and 40 kHz. The effectiveness of ultrasound in irrigation is determined by its ability to produce cavitation and acoustic streaming. Cavitation is minimized and limited to the tip of the instrument used, while the effect of acoustic streaming is more significant.

Ultrasound creates bubbles of positive and negative pressure in the molecules of the liquid with which it comes into contact. The bubbles become unstable, collapse and cause an implosion similar to a vacuum decompression. Exploding and imploding they release impact energy that is responsible for the detergent effect. It has been demonstrated that ultrasonic activation of NaOCl dramatically enhances its effectiveness in cleaning the root canal space, as ultrasonic activation greatly increases the flow of liquid and improves both the solvent and antibacterial capacities and the removal effect of organic and inorganic debris from the root canal walls.
Ultrasonic activation of NaOCl of 30–60 s for each canal, with three cycles of 10–20 s (always using new irrigant), appears to be sufficient time to obtain clean canals at the end of the preparation phase (Figs. 1 & 2). Ultrasound appears to be less effective in enhancing the activity of EDTA, although it may contribute to better removal of the smear layer. The accumulation of debris produced by mechanical instrumentation in inaccessible areas is preventable by using ultrasonic activation of NaOCl even during the preparation phase. The use of a system of ultrasonic continuous irrigation might therefore be advantageous. It involves the use of a needle activated by ultrasound. With this method, the irrigant is released into the canal and is activated by the action of the ultrasonic needle simultaneously.

Chlorhexidine
A final flush with 2% chlorhexidine (CHX) after the use of NaOCl (to dissolve the organic component) and EDTA (to eliminate the smear layer) has been proposed to ensure good results in cases of persistent infection, owing to its broad spectrum of action and its property of substantivity. However, the use of CHX is hindered by the interaction between NaOCl and CHX, which tends to create products that may discolor the tooth and precipitates that may be potentially mutagenic. For this reason, CHX should not be used in conjunction with or immediately after NaOCl. This interaction can be prevented or minimized by an intermediate wash with absolute alcohol, saline or distilled water.

Activation systems
Mechanical instrumentation alone can reduce the number of microorganisms present within the root canal system even without the use of irrigants and intracanal dressings, but it is not able to ensure an effective and complete cleaning. Irrigating solutions without the aid of mechanical preparation are not able to reduce the intracanal bacterial infection significantly. For these reasons, today research is oriented toward the study of systems that can improve root canal disinfection through mechanical activation of endodontic irrigants, and in particular NaOCl. Multiple agitation techniques and systems for irrigants have been used over time, demonstrating more or less positive results.

Manual agitation techniques
The simplest technique of mechanical activation of irrigants is manual agitation, which can be performed with different systems. The easiest way to achieve this effect is to move vertically an endodontic file that is passive in the canal. The use of the file facilitates the penetration of the irrigant, leads to a more effective delivery of irrigant to the untouched canal surfaces and reduces the presence of air bubbles in the canal space, but does not improve the final cleaning. Another similar technique moves vertically a gutta-percha cone to working length with the canal filled with irrigant. Even this method, however, has not been found to improve the intracanal cleaning. For this purpose, in each case, well fitting gutta-percha cones (increased taper) were more effective than cones with the standard taper (0.02). The use of endodontic brushes and of particular needles for endodontic irrigation with bristles on their surface is another technique suggested in order to move the irrigant more effectively within the canals. These systems have been shown to be valid in the removal of the smear layer from root canal walls and thus they can be recommended during irrigation with EDTA to improve their efficacy at the end of the preparation.

Machine-assisted agitation systems
The evolution of manual systems led to the introduction of instruments that can be rotated in handpieces at low speed inside the canal filled with irrigant. They are rotary brushes too large to be brought close to the working length; thus, they can be used effectively only in the coronal and middle thirds of the canal. Other similar instruments are files in plas-
tic with a smooth surface and increased taper or with a surface with lateral plastic extensions, that have dimensions appropriate to achieve the working length if used after the canal preparation. Studies on these systems have shown conflicting results. In general, the results are better than with hand irrigation with a syringe, but lower than that of other more effective systems.16

Continuous irrigation during instrumentation

Recently, a new system for root canal preparation has been introduced to the market. This system uses a particular instrument with an abrasive surface that enlarges the canal via friction in a vibrating motion and allows irrigant to flow through the file itself. This system has shown excellent results in terms of respecting the anatomy and cleaning of difficult root canal anatomies, such as difficult isthmuses, oval canals or C-shaped canals.18 The low cutting efficiency of this system in some cases may limit its use in root canal preparation, but makes it an excellent additional technique to enhance the cleaning and disinfection of the root canal system at the end of the preparation.20 The concept of continuous irrigation was developed in the past with the use of mechanical instruments for sonic and ultrasonic preparation that could concurrently clean through the continuous release of irrigant. These techniques were then abandoned for various reasons related to the poor quality of the preparation itself.

Sonic activation

Sonic activation has been shown to be an effective method for disinfecting the root canals. The recent systems use smooth plastic tips of different sizes activated at a sonic frequency by a handpiece. The system seems to be able to clean the main canal effectively, to remove the smear layer and to promote the filling of a greater number of lateral canals.17 Another recently introduced technique uses a syringe with sonic vibration that allows the delivery and activation of the irrigant in the root canal simultaneously. Sonic activation differs from ultrasonic activation in that it operates at a lower frequency (1–6 kHz), and for this reason it is generally found to be less effective in removing debris than are ultrasonic systems.17,21,22

Apical negative-pressure irrigation

As the irrigant must be in direct contact with the micro-organisms and canal walls to be effective, the accessibility of the irrigant to the whole root canal system, in particular in the apical third, is essential. In order to deliver the irrigant into the root canal for the entire length and to obtain a good flow of fluid, apical negative-pressure systems have been introduced that release and remove the irrigant simultaneously.

These systems consist of a macro-cannula for the coronal and middle portions and a microcannula for the apical portion, and the cannulas are connected to a syringe for irrigation and the aspiration system integrated with the dental unit (Fig. 3). During irrigation, a tip connected with a syringe delivers the irrigant to the pulp chamber without the risk of overflow, while the cannula placed in the canal pulls irrigant into the canal, through the aspiration system to which it is connected, and evacuates it through the suction holes. This system is intended to ensure a constant and continuous flow of new irrigant into the apical third safely and with a lower risk of extrusion.21 Most of the studies on this technique have shown that it is very effective at ensuring a greater volume of irrigant in the apical third24 and excellent removal of debris from this area25 and inaccessible areas,26 with results in the majority of cases similar to those of ultrasonic activation techniques.27–29 From a clinical perspective, apical negative-pressure systems can be effectively integrated with ultrasonic irrigation techniques because they act by different mechanisms. They can operate in synergy with the objective to obtain cleaner canals, especially in the apical third and the most inaccessible areas.

Laser activation

The interaction between the laser and the irrigant in the root canal is a new area of interest in the field of endodontic disinfection. This concept is the base of laser-activated irrigation (LAI) and photon-initiated photoacoustic streaming (PIPS) technology.30 The mechanism of this interaction has been attributed to the effective absorption of the laser light by NaOCl. This leads to the vaporization of the irrigant and to the formation of vapor bubbles, which expand and implode with secondary cavitation effects. The PIPS technique is based on the power of the Er:YAG laser to create photoacoustic shock waves within the irrigant introduced into the canal. When it is activated in a limited volume of liquid, the high absorption of the laser in NaOCl combined with the high peak power derived from the short pulse duration employed (50 µs) determines a photomechanical phenomenon.20 A study showed that there was no difference in bacterial reduction achieved by NaOCl activated by laser compared with only NaOCl.31 Another study investigated the capability of LAI to remove a bacterial biofilm created in vitro on the canal walls.32 This study found that it did not completely remove the biofilm from the apical third of the root canal and infected dentinal tubules. However, the finding that laser activation generated a higher number of samples with negative bacterial cultures and a lower number of bacteria in the apical third was a promising result regarding the effectiveness of the technique, and has been confirmed by a more recent study.33
Additional disinfection systems

In addition to the above-mentioned systems that were able to activate the endodontic irrigants and to improve their cleaning capability, endodontic research is oriented toward the identification of alternative solutions that could further refine disinfection and assist in the destruction of biofilms and the elimination of micro-organisms. For this purpose, different substances and technologies have been investigated over time with different results.

Photoactivated disinfection

A new method recently introduced in endodontics is photoactivated disinfection. This technique is based on the principle that the photosensitizing molecules (photosensitizer, PS) have the ability to bind to the membranes of the bacteria. The PS is activated with a specific wavelength and produces free oxygen, which causes the rupture of the bacterial cell wall on which the PS is associated, determining a bactericidal action. Extensive laboratory studies have shown that the two components do not produce any effect on bacteria or on normal tissue when used independently of each other; it is only the combination of PS and light that exert the effect on the bacteria.

An endodontic system called light-activated disinfection (LAD) has been developed based on a combination of a PS and a special light source. The PS attacks the membranes of micro-organisms and binds to their surface, absorbs energy from light and then releases this energy in the form of oxygen, which is transformed into highly reactive forms that effectively destroy microorganisms. LAD is effective not only against bacteria, but also against other micro-organisms, including viruses, fungi and protozoa. The PSs have far less affinity for the cells of the body; therefore, toxicity tests carried out did not report adverse effects of this treatment. Clinically, after root canal preparation, the PS is introduced into the canal to working length with an endodontic needle and is left in situ for 60 s to allow the solution to come into contact with the bacteria and spread through any structures, such as biofilms. The specific endodontic tip is then inserted into the root canal up to the depth that can be reached and irradiation is performed for 30 s in each canal (Fig. 4). This technique has proven to be effective in laboratory studies at eliminating high concentrations of bacteria present in artificially infected root canals. Care should be taken to ensure maximum penetration of the PS, since it is important that it come into direct contact with the bacteria, otherwise the effect of photosensitivity will not occur. In addition, LAD appears to be effective not only against the bacteria in suspension, but also against biofilm. Research is now directed toward evaluating the possibility of increasing the antibiofilm effectiveness of LAD, combining the benefits of photodynamic therapy with those of bioactive glasses and nanoparticles, which will be described later. Currently LAD is not considered as an alternative, but rather as a possible supplement to standard protocols of root canal disinfection already in use.

Laser

One of the main disadvantages of the current endodontic irrigants is that their bactericidal effect is limited primarily to the main root canal.
In the endodontic field, several types of lasers have been used to improve root canal disinfection: the diode laser, carbon dioxide laser, Er:YAG laser and Nd:YAG laser. The bactericidal action of the laser depends on the characteristics of its wavelength and energy, and in many cases is due to thermal effects. The thermal effect induced by the laser produces an alteration of the bacterial cell wall that leads to changes in osmotic gradients up to cell death. Some studies have concluded that laser irradiation is not an alternative, but rather a possible supplement to existing protocols to disinfect root canals.\textsuperscript{36} The laser energy emitted from the tip of the optical fiber is directed along the canal and not necessarily laterally toward the walls. In order to overcome this limitation, a new delivery system of the laser was developed. The system consists of a tube that allows the emission of the radiation laterally instead, directed through a single opening at its terminal end. The objective of this modification was to improve the antimicrobial effect of the laser in order to penetrate and destroy microbes in the root canal walls and in the dentinal tubules. However, complete elimination of the biofilm and bacteria has not yet been possible, and the effect of the laser has been found to be less relevant than that of the classical solutions of NaOCl.\textsuperscript{37} In conclusion, strong evidence is not currently available to support the application of high-power lasers for direct disinfection of root canals.\textsuperscript{28}

Alternative antibacterial systems

**Nanoparticles**

Nanoparticles are microscopic particles between 1 and 100 nm in size that have antibacterial properties and a tendency to induce much lower drug resistance compared with traditional antibiotics. For example, nanoparticles of magnesium oxide, calcium oxide or zinc oxide are bacteriostatic and bactericidal. They generate active oxygen species that are responsible for their antibacterial effect through electrostatic interaction between positively charged nanoparticles and negatively charged bacterial cells, resulting in accumulation of a large number of nanoparticles on a bacterial cell membrane and a subsequent increase in its permeability associated with the loss of its functions. Nanoparticles synthesized from powders of silver, copper oxide or zinc oxide are currently used for their antimicrobial activity. In addition, nanoparticles can alter the chemical and physical properties of dentin and reduce the strength of adhesion of bacteria to the dentin itself, thus limiting recolonization and bacterial biofilm formation. In any case, the possible success of the application of nanoparticles in endodontics will depend essentially on the manner in which they can be delivered in the most complex root canal anatomy.

**Bioactive glass**

Recently, bioactive glass or bioactive glass-ceramics have been a subject of considerable interest for endodontic disinfection owing to their antibacterial properties, but conflicting results have been obtained.\textsuperscript{5}

**Natural plant extracts**

A current trend is the use of natural plant extracts, taking advantage of the antibacterial activity of polyphenolic molecules generally used for storing food. These compounds have been found to have poor antibacterial efficacy, but several demonstrate significant ability to reduce the formation of biofilms, although the mechanism by which this occurs is not clear.\textsuperscript{5}
Noninstrumentation techniques

The first trial of a method of cleaning without canal preparation was the noninstrumentation technique conceived by Lussi et al. This technique did not provide for the enlargement of the root canals because there was no mechanical instrumentation of the root canal walls. In fact, root canal cleaning was exclusively obtained with the use of NaOCl at low concentration, introduced and removed from the canal using a vacuum pump and an electric piston that created fields of alternating pressure inside the canal. These caused the implosion of the produced bubbles and hydrodynamic turbulence that facilitated the penetration of NaOCl into the root canal ramifications. At the end of this procedure, the canals were filled with a cement conveyed by the same vacuum pump. This system did not prove to be of substantial effectiveness and was never marketed.

Recently, a method has been developed for cleaning the entire root canal system through the use of a broad spectrum of sound waves transmitted within an irrigating solution to remove pulp tissue, debris and micro-organisms quickly. One study showed that this technique was able to dissolve the tissue tested at a rate significantly higher than that of conventional irrigation. More research is needed to determine whether this approach is effective in the root canal system with minimally invasive or no canal preparation.

Conclusion

According to current knowledge, endodontic pathology is an infection mediated by bacteria and in particular by biofilm. From a biological perspective, endodontic therapy must then be directed toward the elimination of microorganisms and the prevention of possible reinfec-
tion. Unfortunately, the root canal system, with its anatomical complexity, represents a challenging environment for the effective removal of bacteria and biofilm adherent to the canal walls. Chemomechanical preparation involves mechanical instrumentation and antibacterial irrigation, and it is the most important phase of the disinfection of the endodontic space. The technological advances of instruments have brought significant improvements in the ability to shape the root canals, with fewer procedural complications. In the management of the infected root canal system, various antimicrobial agents have been employed. Furthermore, some clinical measures, such as an increase in apical preparation and a more effective system of irrigant delivery and activation of irrigant, can promote and make more predictable the reduction of intracanal bacteria, especially in complex anatomical and noninstrumented portions of the root canal system.

Editorial note: A list of references is available from the publisher.

about

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