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General introduction

Challenges of root canal instrumentation

Microbes and their by-products play a decisive role in the onset and continuance of pulpal and periradicular disease (1). The goals of an endodontic treatment (predominantly root canal treatment) are therefore the elimination of microorganisms and microbial components from the root canal system, and the prevention of its re-infection (2-4). Chemomechanical debridement, basically including instrumentation and irrigation is widely recommended in order to achieve these goals.

Although mechanical instrumentation procedures have improved considerably over the years, none of the techniques can completely disinfect the root canal system mainly due to the anatomical complexity of the root canal system (5), leaving up to half of the canal surface untouched following instrumentation (6-9). In addition, the formation of a smear layer after instrumentation, a mixture of dentine debris, pulp tissue remnants, odontoblastic processes and, in infected root canals, microorganisms (10), may affect adversely the elimination of the root canal infection (10-12). Thus, the role of instrumentation has been switched from debridement to providing better access to the apical part for root canal irrigation and filling (13), and irrigation has been playing an increasing role in disinfecting the root canal system.

Irrigation

The objectives of irrigation of the root canal are to eliminate microorganisms, flush out debris, dissolve organic tissue remnants and lubricate instruments (14), especially to debride those areas left unprepared by mechanical instrumentation (13). There are two essential aspects involved in root canal irrigation: the irrigant and the irrigation system.

Irrigants

Ideally root canal irrigants should be biologically compatible and antibacterial, demonstrate good surface wetting, have no adverse effects on remaining tooth structure, and be easy to use and effective within clinical parameters (13). The chemical effect of the irrigants has been studied extensively. Although many kinds of irrigants have been introduced, sodium hypochlorite (NaOCl) is still the most popular one with two distinguished properties: wide antimicrobial spectrum and unique capacity to dissolve organic (including necrotic) tissue remnants (11, 15). The tissue dissolving potential is indispensable, because the necrotic and infected tissue remnants, located in the areas which have not been instrumented, need at least come in contact with the irrigant.

Irrigation Techniques

Apart from the chemical merits of irrigants, four mechanical aspects are important for the irrigation procedure: the dispersion and refreshment of the irrigant in the root canal, mechanical cleaning of the root canal wall and the removal of matter from the root canal. As the irrigant should be in close contact with the microorganisms and tissue remnants to be effective (16, 17), and the irrigant could be inactive shortly after its reaction, the accessibility of the irrigant to the whole root canal system and further refreshment of the irrigant are essential (18, 19). Furthermore, the detachment of biofilm, debris and smear layer by the irrigant from the root canal requires a certain shear stress on the root canal wall by the flow of the irrigant. Finally, the flush action by the irrigant carrying away the loosened debris from the root canal is very essential. All these points are considered as the mechanical actions of irrigation procedure, the key to successful irrigation and root canal disinfection.

In order to enhance the above mentioned mechanical aspects of the irrigation procedure, various irrigation techniques have been developed (Table 1-1).

All the irrigation techniques basically involve the irrigant delivery with or without irrigant activation. Syringe irrigation is the conventional technique and is still widely used. It delivers irrigant into the root canal and carries away the debris out of the root canal by flushing action. It allows good control of the depth of needle penetration within the canal and the volume of irrigant that is flushed through the canal (20). The needle type and size, the shape of the root canal, the flow rate are the essential factors for syringe irrigation effectiveness (21-23).

Combinations of syringe irrigation to deliver the irrigant in the root canal and various ways to activate the irrigant, established many kinds of irrigation techniques. These techniques are applied mainly as final irrigation procedures after the root canal instrumentation is completed. Various irrigant activation techniques

have been proposed to improve the efficacy of irrigation solutions, including activation with syringe needles, hand files, gutta-percha cones, plastic inserts, and (ultra)sonic or laser devices. Syringe irrigation and irrigant activation are performed alternatively in these techniques; therefore the flush method is always intermittent.

Irrigant extrusion from the foramen might result in damage to the periapical tissues. In order to improve the safety of the irrigation procedure, so-called negative-pressure systems have been introduced. Normally, the irrigant is delivered deep into the root canal, usually through a needle, and then flows coronally along the outside of the needle, which is called positive-pressure technique. The pressure difference causing the irrigant flow is created between a pressurized container (e.g. a syringe barrel) and the root canal, where the pressure remains close to atmospheric. In the negative-pressure techniques, the pressure difference is created between the injection cannula positioned coronally and the aspiration cannula placed deep inside the root canal. The irrigant flows at a nearly atmospheric pressure near the canal orifice towards the apex and evacuated by the suction cannula located apically (24, 25).

There are also some concomitant instrumentation and irrigation techniques. Irrigant is delivered through the handpieces around the instruments and root canal instrumentation is performed simultaneously.

Passive Ultrasonic Irrigation

Ultrasonic devices have been used in Endodontics for over half century since its first introduction by Richman (26). Initially, it was used to drive files for root canal preparation and debridement, instrumentation and irrigation was therefore performed simultaneously with a continuous flow of the irrigant. This irrigation technique was called ultrasonic irrigation (UI). This technique became less popular due to 1) the difficulty to control the removal of dentin that potentially produced apical perforations and irregularities (27, 28); 2) the contact between the oscillating file and the root canal wall during the instrumentation constrained the file oscillation and consequently, the acoustic streaming and cavitation would be reduced and as well as the effectiveness of cleaning (29).

Passive Ultrasonic Irrigation (PUI) was first described by Ahmad (29). This irrigation technique was performed with no instrumentation, planing, or contact of the canal walls with an endodontic file or instrument (30) in order to just activate irrigant ultrasonically. This non-cutting technique could reduce the potential to create aberrant shapes on the root canal wall, and was therefore used after instrumentation as final irrigation method.

There are some variations in PUI in terms of the way to deliver the irrigant. (1) Irrigant is delivered by a syringe, and irrigant activation is performed by ultrasonically oscillating file or instrument; the two procedures take place alternatively and the flushing action is therefore intermittent. (2) Irrigant is delivered through the ultrasonic hand piece with continuous flushing (mainly in the pulp chamber), with simultaneous file oscillation and irrigant activation. (3) An ultrasonically oscillating hollow needle attached to the ultrasonic hand piece which will both deliver and activate the irrigant continuously in the root canal.

During PUI, the energy is transmitted from an oscillating file or instrument to the irrigant in the root canal by means of ultrasonic waves. Since the root canal has been shaped and the file was introduced in the center of the root canal trying to avoid any contact with the wall, the file can oscillate freely resulting in the maximum acoustic streaming effect and possible cavitation. The active streaming of the irrigant activated ultrasonically would cause better irrigant dispersion in the root canal and greater shear stress on the canal wall, producing better root canal cleaning efficacy. There is a general consensus that PUI is an effective final irrigation technique in removing pulpal tissue remnants and dentin debris (31-33)

Though PUI seems to be a promising technique as final irrigation, underlying physical mechanisms through which it exerts its efficacy remain to be clarified. In addition, root canal debridement is still a great challenge and PUI improvements are necessary. Therefore a better understanding about the mechanisms of PUI would help us to improve this technique. Some parameters, such as oscillation direction of the ultrasonic file, pulsed ultrasound and ultrasonic intensity, are closely related to the clinical applications and interesting to be studied.

Microbiology studies in Endodontics

Since Kakehashi et al (1) pinpointed bacteria as the causative factor in endodontic disease, a multitude of researchers have performed studies on the alternative irrigants, concentrations of irrigants, combinations of irrigants, and lately various irrigation techniques to optimally eradicate the bacteria from the root canal systems.

The biofilm concept has been recently recognized in endodontic infection. A biofilm, by definition, is 'a microbially derived sessile community characterized by cells that are irreversibly attached to a substratum or interface or to each other, are embedded in a matrix of extracellular polymeric substances that they have

produced, and exhibit an altered phenotype with respect to growth rate and gene transcription' (34). Especially, growing in a biofilm enhances the resistances of the microorganisms significantly (35, 36). Microorganisms do not populate the root canal system in a planktonic state, rather, they grow aggregated on the canal surface producing biofilms (45) that enable the bacteria to resist antimicrobial agents. Microbial behavior in biofilms is rather different from the planktonic state. Therefore, although planktonic killing test and agar diffusion test have a high level of standardization and low costs, they are losing their popularity mainly due to their poor validity. A valid assay for microbial susceptibility testing should be based on a biofilm model. Currently and commonly used biofilm models are discussed later in chapter 10.

Enterococcus faecalis is a Gram-positive coccus from the *Enterococcus* genus. Common reasons for choosing *E. faecalis* in endodontic microbiology studies are 1) this bacteria is commonly isolated in teeth with failing root canal treatments (37, 38); 2) it is known to have several virulence factors that make it difficult to eradicate from the canals. These virulence factors include proton pump mechanisms to maintains optimal cytoplasmic pH levels (39) resulting in the resistance to Ca(OH)_2 (a commonly used root canal dressing material), the ability to withstand prolonged starvation (40), to inhibit the cytokine-producing functions of Th-1 and Th-2 cells (41), to bind collagen (42), and to invade dentinal tubules (43); 3) *E. faecalis* is also easy to grow *in vitro*, and specifically it can form biofilms alone, which allows to study and test bactericidal effectiveness of different agents and techniques on biofilms, though single species. *Streptococcus mutans* is known as one of the causative agents of caries and its cariogenicity has been well established (44, 45). *S. mutans* is able to cause caries mostly due to its ability to form acids and produce biofilm matrix. However, *S. mutans* is also found occasionally in infected root canals with apical periodontitis (46-48). Another reason to select *S. mutans* for our studies was that its biofilm adheres strongly to dentin, which is important for the testing in irrigation studies. Besides, it was also our hypothesis that the interaction between the microorganisms in the dual species biofilms might alter the biofilm formation and also resistance to disinfectant. Therefore we chose the two bacteria for investigations.

Outline of the thesis

In Chapter 2 the cleaning efficacy and physical mechanisms of a sonic irrigant activation device EndoActivator is described.

The efficacy of passive ultrasonic irrigation (PUI) could be further enhanced through a full understanding of the fluid mechanical processes that occur when the irrigant is activated by the energy of an ultrasonically oscillating instrument. Therefore, the influence of the oscillation direction of the ultrasonic file, the pulsed ultrasound, the ultrasonic intensity and the ultrasonic file insertion depth on the efficacy of PUI to remove dentin debris from the apical root canal in combination with the visualization and characterization of the fluid dynamical aspects were described in Chapter 3, 4, 5 and 6 respectively.

Many devices, tools and techniques have been introduced to the root canal irrigation procedure. In Chapter 7 several irrigation systems were evaluated on their efficacy to remove dentin debris from the apical root canal.

Disinfection of the root canal is a big challenge to current root canal therapy, while information on biofilms is limited in endodontic research. Chapter 8 described the biofilms that were formed when *Streptococcus mutans* grew with or without *Enterococcus faecalis*. Chapter 9 discussed how the resistance to antimicrobial irrigant changes when when *Streptococcus mutans* grew with or without *Enterococcus faecalis* by a fluorescence assay.

Table 1-1. Irrigation techniques

Technique							
Delivery method	Activation method	Machine assisted	Simultaneous Instrumentation	Irrigation phase	Delivery pressure	Flush method	
	-	×	×	I and (or) II	positive	C	
	-	×	×	I and (or) II	Positive	C	
	Needle movement	×	×	II	Positive	I	
	Gutta Percha	×	×	II	Positive	I	
Syringe	Brush	×	×	II	Positive	I	
	Rotary Brush	√	×	II	Positive	I	
	Oscillating Tip	Sonic Ultra-sonic	√	×	II	Positive	I
	Laser	√	×	II	Positive	I	
Ultrasonic	Needle	Oscillating Needle	√	×	II	Positive	C
	Hand-piece	Oscillating Tip	√	×	II	Positive	C
		File	√	√	I	Positive	C; I
NIT	-	√	√	I and II	Positive; Negative	C	
Quantec-E	-	√	√	I	Positive	C	
SAF	-	√	√	I and II	Positive	C	
Rinsendo	-	√	×	II	Positive	C	
Safety irrigator	-	suction	×	II	Positive	C	
IAC needle	-	suction	×	II	Negative	C	
EndoVac	-	suction	×	II	Negative	C	

SAF: Self Adjusting File system; IAC: Intracanal Aspiration Technique; NIT: Non-Instrumentation Technique. Irrigation phase: 'I' stands for 'during root canal preparation'; 'II' stands for 'final irrigation'. Flush method: 'C' stands for 'continuous'; 'I' stands for 'intermittent'

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