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Influencing factors and detection methods of microleakage in root canal therapy

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Abstract: *Root canal therapy is a common and effective method to treat pulp and periapical diseases. Thorough root canal disinfection, good root canal shaping, perfect crown seal and apical seal are the keys to successful root canal treatment. The failure of root canal treatment can be attributed to the microleakage of bacteria and other irritants into the root canal and periapical tissues when the root canal system is not tightly filled. This paper will discuss the influencing factors of microleakage in root canal therapy and the research progress of microleakage detection methods.*

Keywords: *root canal therapy, temporary sealing material, microleakage detection*

1. Introduction

The ultimate goal of root canal therapy is to remove diseased pulp tissue from the root canal system, provide a suitable healing environment, and prevent the occurrence of periapical inflammation[1]. With the rapid development of technology, the root canal system can be more and more clearly visualized, and the root canal preparation and disinfection are gradually becoming perfect. The differences in the sealing effect of the root canal system caused by different materials and methods are also increasingly recognized[2-4]. The closure of root canal system includes apical region closure and crown square closure[5]. If the occlusive material fails to do its job, clinically undetectable bacteria, fluids, molecules, or ions may enter the root canal system through the tiny gap between the tooth and the material, leading to the failure of root canal treatment[6]. Therefore, in clinical treatment, how to avoid apical microleakage is the key to improve the success rate of root canal treatment.

2. Microleakage of the crown

In clinical practice, sometimes root canal therapy cannot be completed at one time, so temporary filling materials should be used to temporarily seal the cavity during the return visit. Its main function is to provide a barrier to prevent bacteria from entering the root canal system and prevent the sealed drugs from leaking into the mouth, so as to maintain the continuous effect of the drugs. Not only in the process of root canal treatment, the sealing of temporary crown filling material also plays an important role in the affected teeth that are not repaired timely after root canal treatment.

The stickiness of bacterial saliva, pH value in the oral environment and chewing power are all related to the occurrence of coronal microleakage. Therefore, effective filling materials not only require a close fit with dental tissue to prevent bacteria and microorganisms from entering the root canal system, but also need to exert anti-microbial ability.

2.1 Influence of temporary filling materials on crown microleakage

Temporary filling materials should seal the root canal system to prevent edge leakage. In addition, they need to withstand chewing pressure, have excellent aesthetic properties, chemical and physical stability, ease of handling, do not affect the adhesion of permanent restorations, and storage stability[7]. At present, zinc oxide eugenol cement, glass ionic cement and resin-based temporary repair materials are used separately or in combination for temporary closure[8]. Thermoplastic materials (such as dental glue) have been used less, and resin temporary sealing materials have been gradually used in clinic.

2.1.1 Zinc oxide

Traditional Zinc Oxide Eugenol (ZOE) has been widely used in clinical practice, but it needs manual modulation. When the homogeneity of the material is subjected to manual operation, it may produce bubbles and so on, resulting in low homogeneity of the material, which further affects its sealing ability. Micro-leakage occurs in the early stage of filling. Gradually replaced by its derived materials. Cavition, Cavit G (W), Coltosol F and other substitute materials of ZOE have been applied more and more widely in clinic, and their main components are mainly zinc oxide. Cavition is a hydrophilic temporary sealing material with good sealing property and does not need manual mixing when used. It has good hydrophilicity and expands its volume when solidified in a humid oral environment. It fits closely with teeth and blocks the microleakage of saliva, bacteria and their metabolites in the oral cavity[9]. Shanmugam compared the bacterial microleakage of Cavit G and IRM by observing the presence or absence of bacteria on sterile cotton balls temporarily sealed in the pulp chamber for 7 days, and found that although bacteria growth was observed in more samples in the IRM group, there was no significant difference between the two groups[10].

2.1.2 Glass ionic cement

Glassionomer cement (GIC) was first developed by Wilson and Kent (1972), whose physical properties depend on changes in chemical composition and powder/liquid ratio, providing a wider field of application for clinical applications. As a special filling material, GIC has caries resistance potential related to fluoride release, biocompatibility and chemical adaptation to dental tissue. However, its weak mechanical properties such as low fracture resistance, hardness and wear resistance also limit its restorative effect on the posterior occlusal functional area[11].

GIC can be divided into the following types: traditional glass ionic cement, mixed glass ionomer cement, resin-modified glass ionomer cement and polyacid modified composite resin (complex), etc. Bahsi et al. compared the microleakage after filling tooth neck defects with the above several types of materials, and found that there was no significant difference in the value of leakage depth at the edge of the prostheses, which can be applied in clinical practice[12].

2.1.3 Resins

Composite resin is the most beautiful and functional material in oral clinical direct repair. Its advantages include: wear resistance close to natural tooth structure; Good edge integrity; Oral repair can be performed directly and/or indirectly with ceramic or composite materials; Low cost[13]. Based on modern composite materials and evolving technology, it has excellent adhesion to the enamel, and increasingly to the dentin, with optical properties that allow it to approximate or even reproduce the color and semi-transparency of natural teeth.

Compared with GIC, resin temporary sealing materials have better performance in terms of hardness, edge extensibility and compressive resistance. Although its sealing effect is not affected by chemicals in the pulp cavity, its polymerization or contraction during curing will cause tension or shear force between the dental tissue and the repair material. Tension is one of the important factors that cause cracks between materials and teeth and microleakage. By comparing the dye leakage depth of resin temporary sealing materials with other materials, Wang Xue et al. found that resin temporary sealing materials Clip F had better sealing effect compared with GIC and finished products temporary sealing materials[14].

2.2 Influence of temporary sealing time on crown microleakage

The temporary sealing time also affects the occurrence of crown microleakage. Temporary sealing materials have a certain time limit and can only temporarily seal the crown rather than be used as permanent filling. Madarati[15] found that it was best to seal the crown with temporary sealing materials only for 12 weeks, and the longer the time, the more serious the microleakage; Srivastava's bacterial penetration experiment had similar results. Therefore, the clinical stage of root canal sealing should be less than 2 weeks, and permanent filling or post-crown repair should be performed as soon as possible after the completion of root canal therapy to improve the long-term efficacy of root canal therapy[16].

3. Microleakage of root tips

3.1 Influence of root canal preparation instruments on apical microleakage

The structure of the root canal system is complex, and root canal preparation is a key step in root

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canal therapy. As an important tool of root canal preparation, root canal preparation instruments may affect the apical microleakage. The main instruments of modern root canal preparation are nickel-titanium instruments, which have the advantages of large elasticity, good flexibility, strong forming ability and high cutting efficiency. The Pro Taper Next produced significantly less root canal offset during root canal preparation than the Reciproc reciprocating motion nickel-titanium instrument, and the incidence of apical microleakage was low[17]. In the process of root canal preparation, stress is concentrated on the inner wall of the root canal, resulting in tiny cracks in the dentin of the inner wall, where bacteria, microorganisms and their metabolites gather, leading to the occurrence of root canal microleakage[18].

3.2 Influence of flushing solution on apical microleakage

Due to the complexity of the anatomical structure of the root canal system, it is impossible to achieve thorough disinfection of the root canal using only mechanical preparation technology. Root canal flushes are an important addition to the prep instruments because they remove or wash away bacteria, debris, and necrotic tissue from the c canal[19]. Studies have shown that the stain layer can not only breed bacteria, but also act as a barrier between the sealing material and the wall of the root canal, preventing the penetration of the root canal sealer into the dentin tubules to reduce the permeability of the dentin, which may affect the formation of a tight seal[20].

Clinically used rinsing solutions include: EDTA, citric acid (CA), sodium hypochlorite (NaCLO), chlorhexidine (CHX), etc. EDTA and CA have chelation, which is characterized by the ability to remove the taint layer without changing the structure of the original collagen fiber and increase the exposure of the dentin tubules deep in the root canal, so that the micromechanical combination of the root canal sealant and the dentin tubules can be enhanced to reduce the occurrence of microleakage [21]. The effect of CA is slightly stronger than EDTA at the same concentration. However, Sreedev[22] compared the effect of root canal disinfection using EDTA and CA on the penetration depth of root canal sealers in dentin tubules, and the results showed that the penetration depth of the sealers caused by EDTA was deeper than that of CA. NaCLO is the most commonly used rinsing agent in clinical root canal therapy, which can effectively antibacterial, but its effect on the removal of inorganic parts in the stain layer is weak, and it can also denature collagen fibers, prevent the penetration of sealer into the dentin tubule and the formation of mixed layer, and increase the possibility of microleakage[23]. CHX has good biocompatibility and antibacterial effect. The effective concentration of CHX can disinfect the root canal without damaging the organic matrix of the dentin tubule.

3.3 Influence of sealer on apical microleakage

The root canal sealer is between the inner wall of the root canal and the gum, which is an indispensable part of the root canal filling. In recent years, the types of occlusive agents have been developing and many new occlusive agents have appeared. Traditional zinc oxide clove oil is rarely used at present. Resin Root canal sealers, such as AH Plus, are the most widely used in clinical practice. Bio-ceramic root canal sealers (i Root SP) and silicone root canal sealers (Gutta Flow 2) are emerging root canal sealers. AH Plus has good antibacterial, fluidity and adhesion, and is closely combined with dentin. It has good apical sealing effect, but has certain irritation to periapical tissues[24]. iRoot SP can induce bone tissue regeneration and bioseal apical foramen[25]. There is no shrinkage during solidification, and the possibility of apical microleakage is reduced after filling. Gutta Flow 2 not only does not dissolve after curing, but also exhibits volume expansion of 0.2%[26]. The expansibility of Gutta Flow 2 ensures tight filling of the root canal after the sealer is hardset, avoiding apicular microleakage due to curing shrinkage. The zero solubility of Gutta Flow 2 can guarantee the long-term effect of treatment and reduce the incidence of long-term apical microleakage.

3.4 Influence of filling technology on apical microleakage

The commonly used root canal filling methods include lateral compression of cold glue, vertical compression of hot glue, hot glue of solid core carrier and flow glue of normal temperature. The results showed that the apical microleakage caused by the hot glue vertical filling method was less than that by the cold glue lateral pressure filling method, and the apical microleakage caused by the normal temperature flow glue filling method was less than that by the hot glue vertical filling method[27].

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4. Detection and evaluation of microleakage

At present, dye leakage method, microbial infiltration method, fluid filtration method, electrochemical method, glucose leakage method, scanning electron microscope[28] are different detection methods used to evaluate dental microleakage. Due to the different theories of these methods, their results cannot be compared with each other. Therefore, it is necessary to standardize them in order to more accurately assess the microleakage phenomenon between dental tissue and filling materials.

4.1 Dye leakage method

Dye leakage method was proposed by Grossman. It refers to infiltrating dye into the space between the root canal wall and the filling material or the filling material itself, and evaluating the occurrence of microleakage through observation of dye. Due to its simple operation, it is the most commonly used in vitro detection method at present[29]. After dye penetration occurs, a linear method can be used to observe dye leakage depth by means of longitudinal section, transverse section and transparent tooth method[30]. The disadvantage of the longitudinal cutting method is that it is very unlikely that the cut surface coincides with the deepest penetration of dye, and the data results are unreliable due to the underestimation of leakage.

Commonly used dyes include eosin, methylene blue, Indian ink, basic fuchsin, gentian purple, fluorescent dye[31], silver nitrate, etc. The molecular size, pH value and chemical reactivity of dye particles determine its permeability. Some dyes have lower molecular weights than bacterial toxins. India inks (particle diameter $\leq 3\mu m$) are smaller in weight and size than the bacterial components isolated from the root canal, so they may not accurately represent the size of the actual permeable fluid and microorganisms in the clinic, resulting in false positive results from leakage tests.

4.2 Microbial infiltration method

Human oral cavity is a complex micro-ecological environment with a wide variety of natural flora, and the microbial flora varies greatly in different parts. Bacteria and microorganisms can better reflect clinical relevance than dyes and other substances. Therefore, Goldman et al. proposed the microbial leakage method[32]. There are three methods for detecting microbial leakage: two-compartment leakage model, scanningelectron micro-scope (SEM) and polymerase chain reaction (PCR). The most commonly used two-compartment leakage model is to disinfect the leakage device before the experiment. The filling crown is the culture medium containing bacteria, the root tip is the sterile medium, and the tooth body except the root tip 2 mm is closed. Turbidity of the sterile medium indicates microleakage[33].

Common tracer bacteria include Enterococcus faecalis[34], Streptococcus mutans, Staphylococcus epidermidis, Prevotella melanogenes, Lactobacillus acidophilus, actinomyces odontoides, pseudomonas fluorescens, Clostridium nucleatus, Candida albicans, etc., which can be single bacteria or mixed bacteria, but conclusions vary with different bacterial species. The use of saliva for microbial leakage experiment can stimulate the growth and propagation of residual bacteria in the root canal and better simulate the clinical environment of leakage of single bacteria or mixed bacteria, but it cannot simulate changes in temperature and saliva flow.

Microbial infiltration method is a qualitative experiment, which can simulate the clinical and biological environment well. The size and biological activity of bacteria and microorganisms are related to the pathophysiological activities of the oral cavity. The product can penetrate into the tiny void that bacteria cannot reach, and the product can enter the root canal system before bacteria. As long as bacteria passed through the closed root canal, they would multiply in the medium and cause turbidity, but the infection time of the root tip could not be estimated. In addition, materials with antibacterial properties (such as eugenol paste) cannot be measured by microbial method[29].

4.3 Fluid filtration method

Fluid filtration measures sealing capacity by the movement of bubbles in a closed microtube. The crown of the tooth is connected to a water-filled tube at atmospheric pressure, and the tip of the tooth root is connected to a glass microtube with a uniform diameter of 170 mm and a capacity of 20μl and filled with water. By applying a certain pressure to the crown to force water through the micro-gap between the tooth tissue and the filling material, microleakage was calculated according to the distance of bubble movement in the micro-tube below and the formula of fluid power source[35]. Travel distances

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are observed using a computer-controlled diode laser beam and measured using a computerized fluid filter consisting of a laser system[36].

Compared with dye infiltration method, this method has many advantages. It not only preserves the sample completely, but also eliminates related factors such as dye molecular size, affinity with teeth and pH when measuring microleakage. Since the samples are preserved, the test is repeatable, and the quantitative measurement can be repeated to prevent the operation errors of researchers[37]. In addition, it can be more sensitive to detect voids in the sample, but does not reflect the location of microleakage, and the liquid can be balanced in the voids, resulting in less filtration. Up to now, there is no uniform standard for the pressure and measurement time used in this method.

4.4 Electrochemical method

When a metal comes into contact with a corrosive substance, corrosion occurs through an electrode reaction (an electrochemical reaction). Jacobsen and Fraunhofer[38] carried out electrochemical microleakage experiments based on the diffusion of ions in space. The crown and the root tip were immersed in two separate electrolyte solutions, and the electrodes in the electrolyte were connected to the voltage regulator. The current through the filling material was measured by galvanometer and the sample impedance was obtained according to Ohm's law. The microleakage is inversely proportional to the impedance value and proportional to the current size, which is proportional to the contact area between the electrolyte and the electrode.

Electrochemical method is a quantitative experiment with non-destructive samples, which can be used to measure the same sample for many times for a long time, and to evaluate the influence of thermal cycle, aging, mechanical stress, etc., on the tooth filling interface[39]. However, the composition of electrolyte, the type of electrode, the distance between electrodes and the thickness of electrodes have influence on the experimental results. The accumulation of corrosion deposits on the electrode obstructs ion flow diffusion and affects the accurate expression of current value.

4.5 Glucose leakage method

Glucose is hydrophilic and chemically stable, and it is a nutrient required by bacteria and microorganisms. It can make the bacteria remaining in the root canal multiply, and has good clinical relevance. Xu[40] used glucose as the tracer to analyze the microleakage of the root canal. The crown of the filling was a glucose solution. Glucose molecules penetrated into the root canal through hydrostatic pressure, and the apical solution was extracted and measured with a spectrophotometer. The glucose content of the root canal was filtered, and the glucose concentration was obtained by the glucose oxidase-peroxidase method, so as to calculate the microleakage at different time intervals.

Glucose leakage method is a quantitative experiment capable of continuous non-invasive measurement of microleakage, featuring low cost, high specificity and sensitivity, and is not affected by subjective factors of the observer[41]. However, there are some problems during operation, such as difficulty in maintaining sterility, water consumption and evaporation of glucose, and possible leakage of glucose into dentin rather than root tip.

4.6 SEM

SEM is the most commonly used tool for detection and analysis of solid objects. It transmits electrons to the sample by focusing electron beam, and detects secondary electrons emitted in the sample according to the interaction between electrons and atoms in the sample. After collecting secondary electrons, the probe converts them into electrical signals of different strengths, which can obtain various physicochemical properties of the sample under test and form a complete image[42].

SEM is a qualitative method, which is often combined with dye leakage method to observe the degree of leakage. The acquisition speed is fast, the magnification ratio is large, the image resolution is high, the sample damage and contamination degree is small, the sample surface can be three-dimensional imaging from multiple angles. However, special equipment and vacuum conditions are required. Samples should be dehydrated and dried before detection, otherwise cracks may occur between filling materials and microleakage may be affected.

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5. Summary

To sum up, based on the understanding of influencing factors of microleakage in root canal therapy, researchers conducted a large number of comparative studies on crown microleakage of temporary sealing materials, root canal preparation instruments and flushing solution, root canal sealing materials and different root canal filling techniques using different microleakage detection methods through a large number of in vitro experiments and different research methods. It provides scientific basis and foundation for the clinical work of root canal therapy. Future research direction is to find an ideal root canal flushing solution that can remove the stain layer without eroding dentin to reduce the impact of microleakage on root canal treatment. Screening suitable temporary sealing materials should not only fit closely with dental tissue but also facilitate clinical operation to reduce the occurrence of crown microleakage [43]. In all aspects of root canal therapy, clinicians should strengthen the sealing of root canal to reduce microleakage and improve the long-term success rate of root canal therapy.

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