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Experimental Methods and Research Progress of Antibacterial Properties of Root Canal Sealers

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Abstract

The most important factors of pulp necrosis and periapical infections are the presence of bacteria and their metabolites. Root canal treatment is performed on teeth with apical inflammation, one of the purposes of which is to eliminate microorganisms within the root canal. After mechanical and chemical preparation, there are still stubborn microorganisms in the root canal, which affect the therapeutic effect. Bacteria can invade the deep part of the dentin tubule, and short-term clinical treatment can not completely remove these bacteria, so the long-term sealing of the root canal with bacteriostatic root canal sealant in the root canal can further reduce the number of residual microorganisms and kill the bacteria in the infected root canal to the greatest extent. As an indispensable part of root canal filling, the antibacterial properties of root canal sealers have always been a research hotspot.

Keywords

Root Canal Sealer; Antibacterial; Root Canal Treatment.

1. Antibacterial Experimental Method

1.1 Agar Diffusion Test (ADT)

Agar diffusion experiment is a commonly used semi-quantitative experimental method to detect the antibacterial properties of materials. It is commonly used to detect the antibacterial properties of the dispersion of blocking agent materials in a water environment. The antimicrobial properties of blocking agents are generally measured by measuring the size of the inhibitor circle on agar plates [2]. However, this method is not suitable for blocking agents where the antibacterial component is insoluble in water, because the experimental principle is to use the diffusion rate and solubility of the active antibacterial component in the blocking agent to determine the antibacterial effect [3]. Some scholars have used ADT and DCT to detect the antibacterial effect of the same root canal sealer (AH-plus) on Enterococcus faecalis, and found that in the ADT experiment, AH-plus has no antibacterial effect on Enterococcus faecalis, while in the DCT experiment, AH-plus can show obvious antibacterial effect [4]. For non-water-soluble root canal sealers, the results detected by the ADT method are not accurate.

1.2 Direct Contast Test (DCT)

Direct contact experiment is a quantitative experimental method that is directly mixed with the blocking agent with the culture of interest. Its experimental results are almost unaffected by the solubility and diffusion of the blocking agent. However, the experiment also has obvious disadvantages, that is, in dct experiments, bacteria are mostly present in planktonic form. The bacteria in the infected root canal are mostly present in the form of biofilms and have a pathogenic effect. Moreover, the root canal system is complex and diverse, and bacteria can exist in the root canal wall, root tip divergence, dentin tubules, lateral branch root canals, inter-tube traffic, etc., which lead to difficulties in root canal infection control, so DCT experiments cannot reflect the complexity of the

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root canal system [5]. Portenier [6] et al. suggest that the susceptibility of Enterococcus faecalis to blockers at different stages should be detected in experiments, as enterococcus faecalis in stages is more susceptible to antimicrobial drugs.

1.3 Root Canal Model of Infection

The determination of antimicrobial properties of blocking agents by root canal infection model method was first proposed by Heling et al. scholars, and the model used in the experiment was the intercepted length of 4 mm bull tooth root [7]. This method uses ex vitro teeth for in vitro root canal filling, which can simulate clinical operations well, but the experiment is susceptible to the influence of filling operation techniques. Modern root canal treatment adopts a standardized operation process, which can avoid the influence of filling operation factors to the greatest extent. Select a single tube of in vivo teeth, under the condition of the same number of preparation numbers and tapers, the single-tip method of filling can try to ensure that the amount of blocking agent used in each sample is roughly equal. Therefore, the use of ex vivo root canal infection model to determine the antibacterial effect of blocking agents is currently an accurate method. But the method also has drawbacks, namely the viable but nonculturable state (VBNC) of bacteria. Natural stress responses by bacteria to starvation, unsuitable growth temperatures, high osmosis concentrations, high oxygen concentrations, or exposure to white light, or processes that have a bactericidal effect on bacteria may result in VBNC status [8]. In the root canal model experiment of infection, when the bacteria are in contact with the root canal blocker for a long time, the antibacterial component in the blocker inhibits the biological behavior of the bacteria, and this stimulation may cause the bacteria to enter the VBNC state.

2. Microbial Selection

2.1 Enterococcus Faecalis

Enterococcus faecalis is a gram-positive bacterium, a normal flora of the human body, often parasitic in the mouth, intestines and vagina, and is also an important pathogen of opportunistic infections in the human body. Its body form is spherical or chain-shaped, without capsular membranes and spores, and it is highly adaptable to the environment and can tolerate a variety of antibiotics. Studies have shown that Enterococcus faecalis is absent or rarely present in the primary infection of the root canal, but is more common in retreatment of the root canal [9] and is one of the important microorganisms for persistent root canal infection and reinfection. The pathogenicity of Enterococcus faecalis is related to its drug resistance, hunger tolerance, and biofilm formation structure[10][11].

Zordan et al.[12] Assessed the activity of the bioceramic root canal blocker TotalFill BC Sealer antibacterial and antibacterial biofilm activity by direct contact assay (DCT) of planktonic bacteria (DCTPC) and direct contact experiments on bacterial biofilms (MDCT) formed in bovine dentin; The results showed that TotalFill BC significantly reduced Enterococcus faecalis in DCTPC. In MDCT, TotalFill BC has a higher anti-biofilm efficacy. Nirupama et al. [13] evaluated the antibacterial activity of root canal sealers iRoot SP, AH Plus, Tubliseal EWT, and EndoRez against Enterococcus faecalis, and the results showed that iRootSP and AH Plus had higher antibacterial activity against Enterococcus faecalis. Studies have also shown [14] that endoREZ blockers alone have no antibacterial effect on bacterial biofilms such as Enterococcus faecalis. Liu et al. [15] added dimethylaminodecethyl methacrylate (DMADDM) to EndoreZ, developed a new antibacterial root canal blocker and determined its antibacterial properties, and the results showed that DMADDM significantly increased the antibacterial activity of EndorREZ. Marashde et al. [16] co-assembled silica and ortenidine hydrochloride (OCT) antibacterial surfactants to synthesize polyphosphate (DSP), added them to EndoSequence BC and AH plus, and determined their antibacterial activity against Enterococcus faecalis, and the results showed that DSP enhanced the antibacterial activity of EndoSequence BC but had no effect on the antibacterial properties of AH plus. Dos Santos et al. [17] Added extracts of plants such as Bixa orellana, Mentha piperita and chrysanthemum (Tagetes minuta) to the resin-based root canal blocker RealSeal, and their antibacterial effects were determined by direct contact tests, and Enterococcus faecalis, Candida albicans and Streptococcus proteus were

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selected as the target species, and the results showed that All three extracts promote the anti-enterococcal effect of RealSeal, and after 24 hours, only the T.minuta and B.orellana groups had antibacterial activity against Streptococcus proteus.

2.2 Candida Albicans

Candida albicans is a fungus that is often found on the surface of the skin, in the mouth, in the vagina and in the digestive system, and is generally found in small numbers in the normal body, does not cause disease, and is an opportunistic pathogenic bacterium. Candida albicans is part of the normal oral microbiota associated with failure of pulp therapy and is thought to be a possible dentin-friendly microorganism [18]. Fungal infections may be associated with persistent apical periodontitis, and Candida albicans predispose to secondary root canal infections. Studies have shown that [19] infection with Candida albicans in the root canal is mostly related to candida dense in saliva. Abdel et al. [20] developed propolis-loaded nanoparticles (ProE-Loaded NP) by incorporating propolis into polymer nanoparticles (NPs) and made root canal sealers to determine their antimicrobial activity. Blocking agents show antibacterial activity against strains of Enterococcus faecalis and Streptococcus amoebrasus and antifungal activity in Candida albicans. Zordan et al. [12] also determined the antibacterial effect of TotalFill BC on Candida albicans by direct contact experiments, showing that TotalFill BC completely eliminates Candida albicans. Erhan et al. [21] Determined the antibacterial effect of root canal sealers on Candida albicans by direct contact, it was found that freshly mixed iRoot SP and MTA Fillapex had antifungal effects, the newly mixed AH Plus Jet completely inhibited fungal growth and showed the highest antifungal activity, while GuttaFlow did not show antifungal activity at any time. Nirupama et al. [22] Also selected Candida albicans as the culture of interest when determining the antimicrobiality of root canal sealers, and the results showed that AH Plus and Tubliseal EWT showed higher antimicrobial activity against Candida albicans. Gurpreet et al. [23] Measured the antibacterial effect of root canal sealers on Candida albicans by agar diffusion, and the results showed that EndoSequence BC blockers had the strongest bacteriostatic ability, and MM-Seal did not have any inhibitory activity against Candida albicans.

2.3 Other Microorganisms

Mohammadi et al. [24] compared the antimicrobial effects of AH-26, white mineral trioxide aggregates (WMTA), and CPM blockers on Staphylococcus aureus and Streptococcus aerobicus after 24 hours and 1 week, and the results showed that all three blockers exhibited antimicrobial activity. At 24 hours and 7 days, AH-26 exerted maximum activity against both species of interest. Tanomaru et al. [25] Evaluated the antibacterial activities of Aroseal, Endo CPM sealer, Sealapex, Sealer26, Sealer26, and Intrafill against Micrococcus luteum, Staphylococcus aureus, Pseudomonas aeruginosa, Candida albicans, and Enterococcus faecalis by agar diffusion, and the results showed that the selected material exhibited antibacterial activity in all strains, and Intrafill had antibacterial activity against all strains except Pseudomonas aeruginosa Acroseal is only effective against Micrococcus aureus and Staphylococcus aureus. Lei et al. [26] By loading bismuth oxide, hydroxyapatite and antibacterial agents, namely Ag3PO4 and ZnO nanoparticles, polyurethane (PU) based nanocomposite blockers (CP-Ag, CP-Zn) were prepared, compared with AH Plus and Apexit Plus blockers, and streptococcus proteophyllus and Staphylococcus aureus were selected as the target strains, and the antibacterial activity was determined, and the results showed that compared with CP-Ag blockers, CP-Zn blockers exhibit longer-lasting antimicrobial activity, outperforming two finished root canal sealers.

3. Conclusion

There is currently no perfect solution for the antimicrobial approach of root canal sealers. Depending on the experimental method, the results of the assay will also vary. Therefore, when determining the antibacterial properties of blocking agents, it is far from enough to use one experimental method alone, different experimental methods have their own advantages and disadvantages, and different methods should be used for experiments to analyze their differences. In 1894 Miller first confirmed the

presence of bacteria in the infected root canal, and with the development of science and technology, in the following 100 years, the study of microorganisms has undergone a process of development from species to pathogenesis. The dominant bacteria in root canal retherapy are mainly Enterococcus faecalis, due to its strong tolerance to the environment, resulting in incomplete removal, so people for the root paste antibacterial research experiments, the most selected is Enterococcus faecalis. However, root canal infection is not a single infection, but rather a variety of mixed bacterial infections with anaerobic bacteria. In the re-infected root canal, in addition to Enterococcus faecalis and Candida albicans, actinomycetes, streptococcus, porphyrinum gingivalis, Clostridium polynucleus and so on are often detected. Bacteria will also affect each other, many studies for the paste single bacterial infection of antibacterial studies, although can not fully reflect the infection state in the root canal, but can compare the antibacterial differences of various blocking agents, has a certain reference value.

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