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Innovative Gel in Endodontic Treatment

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Abstract

Irrigation is a pivotal phase in the correct performance of an endodontic treatment, because it represents an essential support for mechanical instrumentation. The removal of the smear layer occurs through the combined use of instrumentation protocols and an appropriate irrigation methodology. Unfortunately, despite effective instrumentation, some areas such as septa, isthmuses and lateral canals are not easily reachable by irrigating solutions, and consequently bacterial contamination in these areas remains a constant concern. To address this challenge, we considered using a gel composed of hyaluronic acid (generically referred to together with its salts as HAS), nano-micro-structured silicon dioxide (SiO_2) and antimicrobial agents. Hyaluronic acid gives the gel optimal viscosity, reducing the iatrogenic effects of endodontic treatment and promoting tissue repair processes, even inside the microtubules after sealing the root canal.The presence of nano-micro-structured silicon dioxide offers numerous advantages, including the slow release of disinfectant agents, a cleansing action and physical lubrication with micro-abrasion during the opening and reaming phases of the root canal. The combined effect of hyaluronic acid and silicon dioxide ensures excellent adhesion of the gel to the instruments, significantly improving the performance of endodontic treatment.

Finally, the antimicrobial action of the gel is guaranteed by the presence of compounds with a biguanidine structure and colloidal silver, which act synergistically to effectively counteract the microflora present in the root canal and its appendages.

In summary, the use of this innovative gel offers numerous advantages in the endodontic treatment, improving the effectiveness of irrigation and helping to ensure optimal results for the patient's dental health.

Keywords: Endodontic; Root Canal Treatment; Canal Irrigation; Innovative Material

Introduction

Endodontic treatment becomes necessary primarily when a tooth develops apical periodontitis, which is often caused by a bacterial infection characterized by highly structured and resilient mature oral biofilms.

Apical periodontitis is an inflammatory condition of the tissue surrounding the tooth that leads to jaw bone loss. It occurs when bacteria enter the tooth through a damaged crown or deep decay, infecting the dental pulp and causing painful inflammation at the root of the tooth [1].

Root canal treatment is aimed at removing bacteria and their waste products from the root canal system through mechanical and chemical means. Although the use of mechanical instruments is essential to prepare root canals, it does not ensure complete disinfection. Studies indicate that approximately 35-53% of root canal walls remain intact, it allow to biofilm to persist, smear layers to form, and untreated surface areas to become inaccessible [2].

Modern endodontic treatment procedures aim to save a compromised tooth from infection or deep decay by focusing on cleaning, disinfecting and sealing the root canals within the tooth structure. However, the complex anatomy of the root canal system can harbor microorganisms, posing challenges for effective decontamination difficult. In oval canals only 40% of the apical wall area of the root canal comes into contact with the rotary file [3]. In conventional practice, the focus is on removing infected dentin and disinfecting the canals using an irrigant fluid. However, traditional manual irrigation, which uses a needle and sodium hypochlorite solution, presents difficulties in ensuring effective cleaning of the apical area and can be hindered by the accumulation of air bubbles in the canal. To overcome these obstacles, more advanced methods have been developed, such as the use of ultrasound, sonic oscillations and laser techniques, which allow better diffusion of the irrigating liquid within the root canal system.

Although difficult, endodontic treatment is an effective method to save a severely damaged tooth, allowing the patient to maintain his natural dentition and avoid extraction. Well-performed endodontic treatment can provide pain relief, restore tooth function, and improve long-term dental health [4]. It has been established that the chances of complete healing in teeth affected by apical periodontitis are 10-15% lower than in unaffected teeth [5].

Irrigation is considered the most important phase of endodontic treatment as it allows a significant reduction in the bacterial load in the root canal system. During and after instrumentation, irrigating solutions facilitate the eradication of microorganisms, necrotic tissues and dentinal debris. Furthermore, before irrigation, the friction between the instrument and the dentin is reduced, the cutting effectiveness of the files is improved and the correct cooling of the canal is ensured. Irrigation prevents the compaction of tissue residues in the apical portion of the root canal and the extrusion of planktonic bacteria and biofilm into the periapical tissues [6]. The irrigating solutions available on the market today must possess a valid ability to dissolve organic and inorganic tissues, along with demonstrating good antimicrobial activity and a low degree of cytotoxicity. An optimal irrigant solution should exhibit the following characteristics: low cost, strong antimicrobial action, preservation of instrument cutting efficiency, temperature control, ease of dissolution of organic and inorganic material, effective penetration throughout the entire root canal system, non-cytotoxicity, hypoallergenicity , absence of interference with other materials used during root canal therapy and, finally, preservation of dentine.

In current clinical practice, it is essential to use a combination of two or more irrigating solutions in a specific sequence to contribute to the success of root canal treatment. Therefore, an ideal irrigation practice should be efficient in both chemical and physical disinfection by contributing to the detachment of biofilm and planktonic bacteria and exerting shear stresses on the root canal wall.

The aim of this work is to develope an irrigant solution capable of effectively penetrating the root canal system without causing accidental damage. This goal aims to ensure effective treatment by eliminating the risks associated with iatrogenic damage, thus enabling safer and more efficient therapy for dental health.

Materials and Methods

Gel Composition

The gel we performed and tested consists of Hyaluronic acid, Nano-micro-structured silicon dioxide (SiO_2), Antimicrobial agents. Hyaluronic acid and its salts (HAS) used in the gel have a molecular weight between 50 and 3,000 kDa. Nano-microstructured silicon dioxide (SiO_2) refers to a hydrophilic silica particle with a size between 5 and 50,000 nm. Antimicrobial agents are compounds with a polymeric biguanidine structure such as polyhexamethylene biguanide (PHMB) and polyaminopropyl biguanide (PAPB).

Cell Culture and Treatments

The immortalized human keratinocyte cell line HaCaT was grown in DMEM including 2mM l-glutamine that was supplemented with 100 U/mL penicillin and 100mg/mL of streptomycin, 1% non-essential aminoacids (NEAA), and 10% heatinactivated fetal bovine serum in a humidified atmosphere (95% air / 5% CO2) at 37◦C. The cultures were used for all experiments when grown to sub-confluent monolayers.

HaCat cells were seeded into 96-well plates until reaching 80% confluence and then incubated for 6 and 24 hours with the gel. At the end of this incubation, resazurin was added at a concentration of 0.5 mg/ml and incubated for 4 hours to perform the Alamar blue test.

The Alamar blue test was used to evaluate the viability of cultured cells. It is based on the transformation of resazurin, which is blue in color, into resofuran, which is intense red in color. Metabolically active cells reduce resazurin by producing resofuran, which fluoresces and changes color from blue to red. Higher concentrations of resofuran indicate a higher percentage of viable cells. The percentage reduction of resazurin (% ABRED) is calculated using a mathematical formula that takes into account the amount of resofuran produced compared to a baseline control.

Antimicrobial Activity on Plate

We examined the antimicrobial activity of the oral gel against the bacterial strain *Enterococcus faecalis*. The broth culture obtained after overnight incubation was diluted to the optical density of 0.5 O.D., and 100 µL of this were plated on an agar plate. Next, we made holes in the plate and added the oral gel. After an overnight incubation at 37°C, we observed the formation of a transparent halo around the holes, suggesting an inhibition of bacterial growth by the gel.

Antibacterial Activity in Liquid Media

The *Enterococcus faecalis* strain, previously grown as described, was diluted to a concentration of 1-5 x 10^8 CFU/ml. Subsequently, it was incubated overnight in 96 multiwell plates containing 100 µL of medium supplemented with approximately 100 µL of gel. The next day, a spectrophotometric reading was performed at 570 nm.

Results

Our formulated gel comprises an essential combination of components, including hyaluronic acid, nano-micro-structured silicon dioxide (SiO_2) and antimicrobial agents. Hyaluronic acid and its salts (HAS) are carefully selected for their molecular weight, which varies between 50 and 3,000 kDa, allowing for a range of low, medium and high molecular weight fractions. This variety of molecular sizes allows the gel to interact optimally with surrounding tissues, facilitating greater effectiveness and versatility in its use. Nano-micro-structured SiO_2, with hydrophilic silica particles ranging in size from 5 to 50,000 nm, offers a hydrophilic substrate that promotes better interaction with the surrounding environment and increases its ability to spread uniformly in the treatment zones. Antimicrobial agents, particularly biguanidine polymers such as polyhexamethylene biguanide (PHMB) and polyaminopropyl biguanide (PAPB), are chosen for their effectiveness in preventing and controlling infections. Their polymeric structure offers greater stability and persistence over time, allowing the gel to maintain its antimicrobial properties even after application. The synergistic combination of these components in our gel offers a complete and versatile solution for a wide range of applications, while ensuring optimal efficacy, safety and tolerability for patients.

To evaluate the efficacy and tolerability of the gel we formulated, we conducted in vitro investigations using HaCat cells. Initially, we analyzed cell viability as a first step. HaCat cells were incubated for 6 and 24 hours with the gel. After 6 hours from application, a cell viability of still 77% was observed compared to control samples, indicating a good degree of tolerability of the gel by the cells (figure 1)

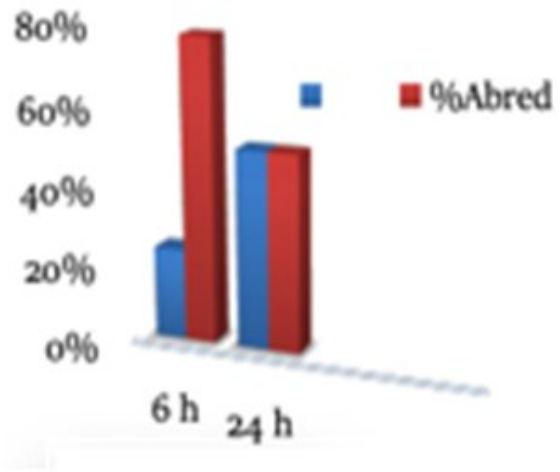

Figure 1: Below are the results obtained, expressed as % viable cells (% ABRED) compared to untreated cells (CTRL)

Subsequently, we performed further tests to evaluate the antimicrobial capacity of the gel both in agar plate and in liquid medium. For the agar plate test, we plated 100 µL of an *Enterococcus faecalis* strain onto agar medium. Next, we made holes in the plate where we added approximately 100 µL of oral gel. After an overnight incubation at 37°C, we observed the presence of a transparent halo around the holes, indicative of growth inhibition of the seeded microorganism. After 24 hours of incubation, a halo of growth inhibition of E. faecalis with an average diameter of 1 cm was observed (Figure 2).

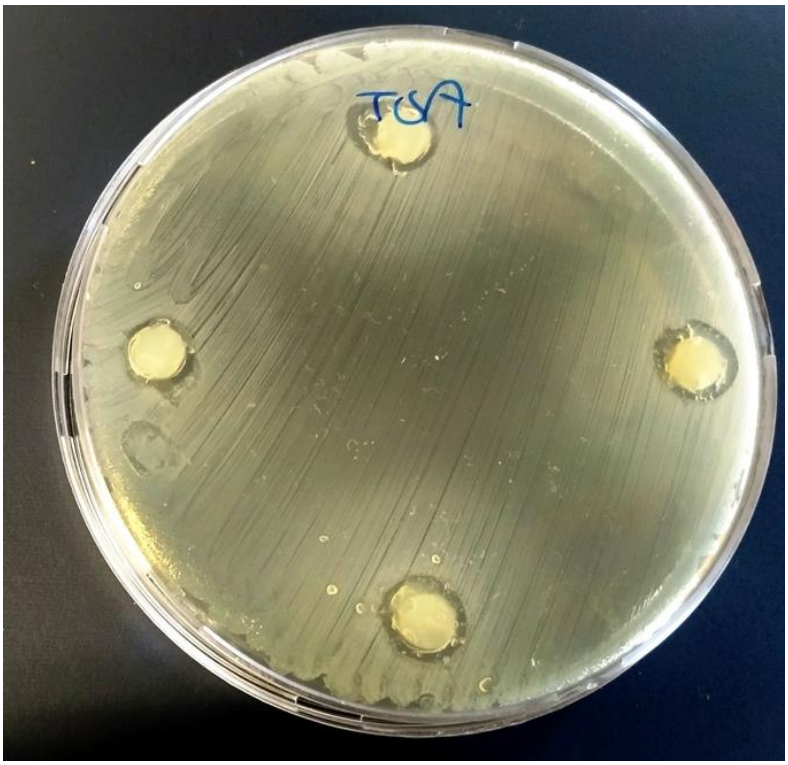

Figure 2: The test, repeated in quadruplicate, shows the presence of a halo of inhibition of the growth of E.faecalis with an average diameter of 1 cm.

The results of the antibacterial activity in the liquid medium confirmed the inhibition of the growth of *Enterococcus faecalis* when our gel was added to the culture medium (Figure 3)

	C-	+gel
TSB	0,06	0,28
E. Faecalis	0,45	0,37

Figure 3: As can be seen, the gel alone with the medium gives the solution a turbidity which causes a higher reading than the blank (TSB only), but bacterial growth is totally inhibited, in fact the addition of E.faecalis does not cause any increase of the O.D. (which instead are higher for E.faecalis alone).

This series of tests provides significant evidence of the effectiveness of our gel in both cellular tolerability and bacterial growth inhibition, suggesting its potential use in clinical applications.

Discussion

Irrigating solutions act as vehicles to transport disinfectant and antimicrobial agents within the root canal system, thus contributing to the eradication of pathogenic microorganisms and their toxic byproducts. Furthermore, these solutions are crucial

for the removal of tissue debris and bacterial residues that may be left after the instrumentation step. Another important aspect is the ability of irrigating solutions to prevent the accumulation of tissue residues and the expulsion of bacteria into the surrounding tissues during treatment. Furthermore, they must be effective in reducing the bacterial load without compromising the cell viability of the surrounding tissues and, they must be able to effectively penetrate the entire root canal system, including access to difficult to reach areas such as septa, isthmus and lateral canals . Irrigation represents a critical and indispensable phase in endodontic treatment, contributing significantly to the overall success of the treatment and the long-term health of the treated tooth.

We have developed an irrigant gel that effectively penetrates the root canal system without causing damage, thanks to an essential combination of components. Among these, hyaluronic acid, nano-microstructured silicon dioxide (SiO_2) and antimicrobial agents play a key role. The compounds with a biguanidine structure and colloidal silver ensure an effective contrast to the microflora that infects the root canal and its appendages.

The gel is non-toxic and is particularly suitable for endocanal treatments without iatrogenic effects. Its rheological, abrasive and bioadhesive properties integrate synergistically with antimicrobial agents to maximize the effectiveness of the treatment.

Hyaluronic acid gives the gel an optimal consistency, reducing the iatrogenic effects of endodontic treatment and promoting tissue repair, even within the microtubules after sealing the root canal. The nano-microstructured silicon dioxide, with its high specific surface area, ensures the slow release of disinfectant agents, acts as a detergent and offers physical lubrication with microabrasion during the opening and reaming phases of the dental canal.

The combination of hyaluronic acid and nano-microstructured silicon dioxide ensures excellent adhesion of the gel to the instruments, significantly improving the performance of endodontic treatment. In conclusion, our irrigant gel represents an advanced and effective option for endodontic treatment, offering numerous advantages for a successful and safe dental practice.

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