Article

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Preventive Mechanism of HAP Repair Coatings on the Enamel Surface on Caries in Rats with Oral Infection

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Background: Dental caries, a prevalent oral disease, has long been a focal point of research in dentistry concerning its prevention and treatment. While hydroxyapatite (HAP) repair coatings and sodium fluoride solutions are commonly employed for combating dental caries, but their effectiveness and underlying mechanisms are not fully understood. This study aims to investigate the preventive impact of hydroxyapatite repair coatings on oral infectious caries in rats.

Methods: To create an oral infectious caries model, a total of 35 Wistar rats were selected. After the selection process, 30 rats successfully were established as the model. These rats were randomly divided into three distinct groups for treatment purposes. The first group was the physiological saline group, where the surface of teeth was gently wiped with cotton soaked in physiological saline. The second group was the sodium fluoride group, in which the surface of the teeth was wiped with cotton soaked in sodium fluoride solution. The third group was the experimental group, where the surface of the teeth, previously corroded by acid, was treated with HAP paste. The treatment was performed once a week for a total of four weeks. To evaluate the effectiveness of each treatment, the colony count and Keyes score were recorded. Furthermore, the morphology of the tooth surface for each group was closely observed using an electron microscope.

Results: Based on the experimental results, the experimental group showed significant improvement after four weeks of treatment. The colony count revealed a significant decrease in the number of *Streptococcus mutans* in the experimental group ($p < 0.05$). Additionally, the Keyes score demonstrated that the experimental group had significantly lower scores in the enamel (E)-level and slight dentinal (Ds)-level on the smooth surface of caries compared to both the sodium fluoride group and the saline group, with statistical significance ($p < 0.05$). At the moderate dentin (Dm)-level, the experimental group's scores were significantly lower than those of the control group ($p < 0.05$). The electron microscopy results showed that the experimental group exhibited a marked enhancement in the surface structure of the teeth. Specifically, the experimental group displayed shallow demineralization areas and a reduced number of cavities compared to the sodium fluoride group and the physiological saline group. Furthermore, the experimental group showed a superior ability to inhibit dental caries, indicating its potential as a promising solution for dental health.

Conclusions: The application of HAP repair coatings has demonstrated a substantial reduction in the population of *Streptococcus mutans* within the oral cavity of rats, with no significant difference in the degree of damage on smooth and cracked surfaces of caries compared to the sodium fluoride group. Electron microscopy observations showed that HAP repair coatings can protect the surface of enamel from acid erosion and abrasion. These results suggest the promising potential of HAP restorative coatings in the prevention of dental caries and provide novel directions for future clinical practice.

Keywords: caries; HAP coating; enamel; caries prevention

Introduction

Dental caries, commonly known as tooth decay, is a chronic and progressive disease that affects the hard tissues of the teeth due to bacterial infection from plaque. This results in the destruction of tooth surfaces, leading to the formation of cavities and defects [1]. Dental caries are caused by bacteria, and there are many types of cariogenic bacteria, mainly *Streptococcus mutans* and *Lactobacillus*. These bacteria mix with mucin and food debris in saliva, adhering to tooth surfaces, pits, and fissures, forming an adhesive film known as dental plaque [2]. Many bacteria within dental plaque produce acid, leading to the decalcification and dissolution of the enamel surface of the tooth beneath the plaque. The shape, structure, and positioning of teeth play a significant role in the development of dental caries. Pits

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and fissures present on the biting surfaces of teeth are developmental defects that can trap bacteria and food debris, making them difficult to remove, thus facilitating the formation of dental caries [3,4]. Insufficient mineralization, especially in teeth with insufficient calcification, results in enamel and dentin that are less dense, offering lower resistance to caries and increasing susceptibility to tooth decay. When dental caries occurs, dental tissue is systematically eroded, gradually damaged, and disintegrated, leading to the formation of cavities. The affected tooth surface may appear visibly blackened, and food particles embedded in these cavities can cause discomfort and pain. Dental caries is a significant contributor to tooth loss and can progress to secondary pulpitis and apical periodontitis, potentially resulting in inflammation of the alveolar bone and jaw [5,6].

Enamel is located on the surface of the tooth and is a highly mineralized tissue composed of hydroxyapatite (HAP). Enamel provides sufficient hardness to the tooth and provides basic functions such as mastication, yet its positioning on the tooth's surface makes it particularly susceptible to dental caries, which often originate in the enamel layer [7,8]. HAP, a key inorganic component of both human and animal bones and teeth, is characterized by its needle-shaped crystal structure, with bone collagen fibres arranged regularly around it. Despite its slight solubility in water, HAP exhibits strong ion-exchanging capabilities, enabling it to adsorb inorganic ions, organic small molecules, and biological macromolecules. HAP can chemically bond with bone, due to its capacity for cell-material adhesion. This adhesion is critical for the migration, differentiation, and proliferation of cells. Porous HAP not only exhibits biological activity but also serves as a bone-guiding role, providing an essential scaffold for the formation of new bone tissue. New bone tissue can be generated at the interface between porous HAP and bone tissue, as well as on the inner and outer surfaces of the material. Due to its good biological activity, biocompatibility, and similarity to natural bone inorganic materials, HAP can form close bonds with human soft and hard tissues in a short time after implantation. Consequently, HAP has been widely used as a bone replacement material. The process of enamel self-repair in the salivary environment is not only slow but also often insufficient to protect or repair the enamel layer effectively. Consequently, tooth erosion can progress, ultimately leading to the destruction of the tooth structure [9]. To address this issue, there has been an urge for the development and application of new biomimetic materials and technologies that aim to restore enamel. Standard dental caries treatment typically involves filling teeth. This process entails removing damaged tissue, shaping the tooth into a specific cavity, cleaning and disinfecting the area, and filling the cavity with materials to restore the shape the tooth's original shape. Notably, primary caries that have not yet formed cavities can still benefit from medical treatment, which can yield positive results [10]. Sodium fluoride paste is commonly employed to treat caries, while silver diamine fluoride can be applied to primary teeth. Despite these treatments, recurrence may still occur, necessitating re-evaluation every half year. However, there are several potential issues to consider: (1) different chemical compositions can result in varying mechanical properties, leading to easier wear; (2) the biocompatibility of certain materials may be suboptimal, such as the toxicity of amalgam or the irritation caused by some resins to the dental pulp; (3) material shrinkage can cause gaps between the tooth tissue and the material, increasing the risk of secondary caries; and (4) the mechanical treatment process may unintentionally remove some healthy enamel, thereby increasing the risk of enamel damage and dentin damage [11–13].

HAP is the main component of vertebrate bones and teeth, and the content of HAP in human enamel is approximately 96 Wt%. This compound boasts remarkable biocompatibility and biological activity, making it a potential candidate for bone or tooth induction [14]. Recent research on HAP composite bone substitute materials has made significant progress. However, only a few of these materials can be used in practice. All HAP composite bone substitute materials exhibit certain limitations. To date, no HAP composite bone substitute material has been developed that can simultaneously offer optimal mechanical compatibility, biocompatibility, osteoconduction, and osteoinduction. While reinforcement techniques such as fiber, whisker, and particle reinforcement can effectively improve the mechanical properties of HAP composites, they do not provide a comprehensive solution. The addition of these substances can also negatively impact the biocompatibility of HAP ceramics. Despite progress, HAP-coated materials still face challenges, including a high elastic modulus and low bonding strength. In addition, the large difference in physical properties, such as thermal expansion coefficient and elastic modulus, between HAP powder and the metal matrix can lead to substantial thermal stress within the coating. Furthermore, the coating on the matrix surface is prone to peeling, dissolution, and erosion, resulting in a significant decrease in the bonding strength between the matrix and the implant. Experimental evidence has demonstrated that HAP particles possess excellent biocompatibility and high affinity with enamel. The mineralizing solution of HAP can effectively form remineralized deposits, increasing the local concentration of calcium and phosphorus ions in the oral cavity. This process prevents calcium loss, addresses enamel demineralization, and serves as a fundamental solution to dental caries [15,16]. A rat model of dental caries infection was established, and HAP repair paste was prepared and applied to the normal saline group, sodium fluoride group, and experimental group. Observation of experimental indicators was conducted to explore the anti-caries effect of the enamel HAP coating and provide a reference for the repair and prevention of dental caries.

Materials and Methods

Experimental Animals

A total of 35 male Wistar rats, aged 21 days and weighing 45.36–54.85 g, free from major zoonotic agents, virulent infectious diseases, and pathogens that could compromise animal welfare or research integrity, were provided by the Affiliated Hospital of Chifeng University Animal Experimental Center (Chifeng, China). This study received approval from the Ethics Committee of the Affiliated Hospital of Chifeng University, adhering to established guidelines for the care (ethics approval number: 20203712), handling, and experimentation involving laboratory animals. At the end of the experiment, all the rats were euthanized by CO_2 inhalation.

Main Reagents, Consumables, and Instruments

Streptococcus mutans NCTC 10449 was provided by the Affiliated Hospital of Chifeng University. The reagents used included deionized water (YSH408-01, Yanjin Biotechnology Co., Ltd., China, Shanghai), $Ca(NO_3)_2{\cdot}4H_2O$ (13477-34-4, Xinrunde Chemical Co., Ltd., Wuhan, China,), $(NH_4)_2HPO_4$ (12132456, Xinyao Biotechnology Co., Ltd., Dongguan, Guangdong, China), concentrated ammonia solution (7664-41-7, Tianzheng Pharmaceutical Accessories Co., Ltd. Xi'an, Shaanxi, China), high-purity H_3PO_4 (7664-38-2, Ronghong Technology Development Co., Ltd., Mianzhu, China), high-purity H_2O_2 (7722-84-1, Sigma Aldrich, Shanghai, China); Tryptone soy broth culture medium (HB4114, Haibo Biotechnology Co., Ltd., Qingdao, China), Procaine penicillin injection for veterinary use (160605, Gongyi Pharmaceutical Co., Ltd., Shanghai, China), 3% pentobarbital sodium solution (57-33-0, Xulijin Biotechnology Co., Ltd., Xiamen, China), and electric heating incubator (60007211, Zhangdong Medical Technology Co., Ltd., Shanghai, China).

Preparation of Streptococcus Mutans Suspension

Streptococcus mutans were revived at room temperature and then inoculated on a clean bench using a sterile loop into a test tube containing tryptone soy broth solid medium. The inoculated medium was incubated anaerobically at 37 °C in an electrothermal incubator for 48 h. A smear examination was performed, and after confirmation of pure culture, a single colony was selected from the tryptone soya broth solid medium plate and transferred into a new test tube containing 5 mL of tryptone soya broth liquid medium. The tube was incubated anaerobically at 37 °C in an electrothermal incubator for 24 h or until the number of colonies reached 1×10^{11} CFU·L^{-1}.

Preparation of HAP

HAP was prepared by mixing 0.5 mol/L $Ca(NO_3)_2$ and 0.3 mol/L $(NH_4)_2HPO_4$. The mixture was then stirred using the ripening method, and the pH value was adjusted to 12 by adding concentrated ammonia. The solution was stirred vigorously for approximately 30 min, allowed to precipitate at room temperature, and incubated for 2 h after the precipitation was complete. The resulting precipitate was then centrifuged, washed, dried at 80 °C, and underwent further processing to obtain the HAP powder.

To prepare the curing solution, 85% H_3PO_4 and 30% H_2O_2 were combined in a ratio of 1:4 to obtain the solution. Then, 10 mL of the solution and 1.5 g of the prepared HPA powder were thoroughly mixed to create the repair paste. After etching the enamel surface, it was placed in the repair paste for approximately 15 min, washed, and dried naturally to obtain a coating on the enamel surface.

Establishment of a Dental Caries Model of Oral Infection in Rats

A rat dental caries model was established [17]. First, oral saliva was cultured from 21-day-old rats to confirm stable *Streptococcus mutans* colonization in the oral cavity, and rats were given water mixed with penicillin (200 mg·L^{-1}) for drinking in 21–23-day-old rats. Oral saliva was cultured at 24 days of age to detect the inhibitory status of oral *Streptococcus mutans*, and the drug was discontinued if the result was negative. During 25–27 days of age, rats were anaesthetized by intraperitoneal injection of a 3% pentobarbital sodium solution (30 mg·kg^{-1}) once daily, and 100 µL of *Streptococcus mutans* (1×10^{11} CFU·L^{-1}) was inoculated into each molar surface in the oral cavity of rats for 3 consecutive days to maintain fasting water within 30 min. Subsequently, rats were provided with cariogenic food and drinking water containing *Streptococcus mutans* bacterial solution. At 28 days of age, 100 µL of saliva was collected from the oral cavity of each rat, and colonization success was considered when plate colony counts indicated bacteria levels $>1 \times 10^{11}$ CFU·L^{-1}.

Animal Grouping and Experimental Methods

After surgery, one rat contracted an infection due to operational complications, which ultimately led to death. Additionally, two rats had to be euthanized due to severe oral bleeding after surgery, which resulted in significant weight loss and a decline in their overall condition. These rats were unable to withstand the stress after surgery, and during the experimental period, two other rats experienced severe symptoms such as breathing difficulties and arrhythmia. Despite timely emergency measures, ultimately, it was not possible to save their lives. Despite these setbacks, we were successful in establishing our model in 30 rats. After the successful creation of the model in these 30 rats, they were randomly divided into three groups: the experimental group, the sodium fluoride group, and the saline group, with 10 rats in each group. The rats were anesthetized by intramuscular injection of ketamine (75 mg/kg), with the addition of dexamethasone if needed to enhance the anes-

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thetic effect and reduce side effects. For upper tooth surgeries, the rats were positioned supine, while for lower tooth procedures, they were placed prone. The mouth of the rats was held open using appropriate tools to expose the molars.

In the experimental group, sterile cotton swabs were utilized to apply HAP restorative to all surfaces of rat molars for approximately 15 min.

In the sodium fluoride group, sterile cotton balls were soaked in 100 μL 2 $g \cdot L^{-1}$ sodium fluoride solution. Each surface of the molars was then gently rubbed with the soaked cotton balls and the solution was applied for 5 min.

For the physiological saline group, sterile cotton balls were soaked in 100 μL physiological saline solution. All sides of the molars were gently rubbed with the soaked cotton balls, and the solution was applied for 5 min.

The procedure was performed once a week for a total of 4 weeks.

Outcome Measures

(1) Bacterial colony count of rat molars. At 28 days of age, the rat molars were prepared by wiping the molar surface with a sterile cotton swab pre-moistened with a disinfectant solution. The swab was then immediately placed in a test tube containing 2 mL of normal saline and thoroughly mixed. The solution was diluted 10× to 10^{-5}, and 100 μL of the suspension was added to tryptone soy broth solid medium. The plates were incubated anaerobically at 37 °C for 48 h, and the number of bacterial colonies per liter was counted. The data were analyzed and processed using SPSS 24.0 (IBM, Armonk, NY, USA) software.

(2) The dental caries status of each group of rats was observed as follows. After the experiment, the rats were euthanized, and their molars were extracted. The extracted molars were cleaned, air-dried, and stained. After staining, the residual dye was washed away. Longitudinal sections of the molars were prepared, and the dental caries status was observed under an optical microscope.

(3) Keyes score of rat molars [18]. After the end of the experiment, the rats were euthanized. Rats were placed in a gas anesthesia chamber where a high concentration of halothane was introduced, inducing gradual deep anesthesia, and then the gas concentration was increased, so that they died safely. Their molars were extracted, washed, and allowed to air dry. The Keyes score of the molars from each group of rats was recorded. The degree of dental caries damage can be categorized into four grades: enamel only (E) (this stage is characterized by dental caries damage occurring solely in the enamel layer; slight dentin (Ds) (at this stage, dental caries damage reaches within ¼ of the enamel and outer dentin layers); moderate dentinal (Dm) (this stage is marked by dental caries damage that has reached ¼ to ¾ of the dentin thickness; and extensive dentinal (Dx) (at this advanced stage, dental caries damage has exceeded ¾ of the dentin thickness and may even extend to full-thickness damage). For each rat in each group, the scores for the same level of smoothness and fissure were summed to produce the total score for that level. Subsequently, the mean Keyes score for each group of rats was obtained by dividing the total score by 10.

(4) Scanning electron microscopic morphology of rat molar tooth surface. The rat molars were immersed in 10% formaldehyde solution for 3 h, washed with a phosphate-buffered saline solution, dehydrated using ethanol, and then treated with hexamethyldisilazane for 5 min. Subsequently, the samples were dried, gold-coated using a small ion sputter instrument and examined under an electron microscope to observe the tooth surface morphology. Three independent observations were made.

Statistical Analysis

All experimental data were statistically analysed using SPSS 24.0 software (IBM, Armonk, NY, USA). Measurement data are presented as mean ± standard deviation ($\bar{x} \pm s$). The repeated measures variance method was used to compare bacterial colony counts, while one-way analysis of variance was used to compare Keyes scores among rats within each group. The Tukey test was used to examine the differences between groups. The significance test level was set at $\alpha = 0.05$.

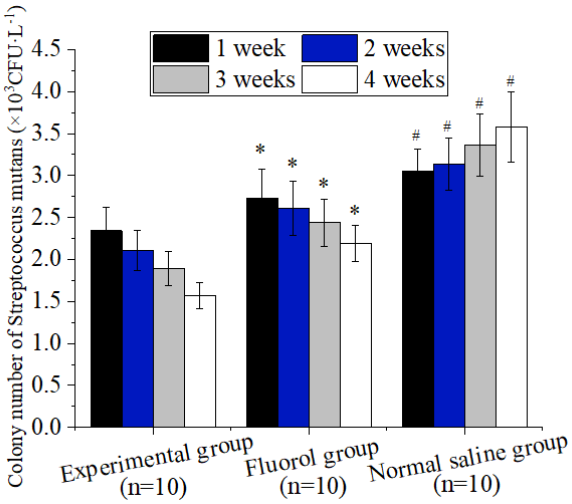

Fig. 1. Comparison of bacterial colony count in molars of rats at different time points in each group. Note: # indicates that the colony count of *Streptococcus mutans* in the molars of the experimental group was remarkably different from that of the saline group ($p < 0.05$); * indicates that the colony count of *Streptococcus mutans* in the molars of the experimental group was remarkably different from that of the sodium fluoride group ($p < 0.05$).

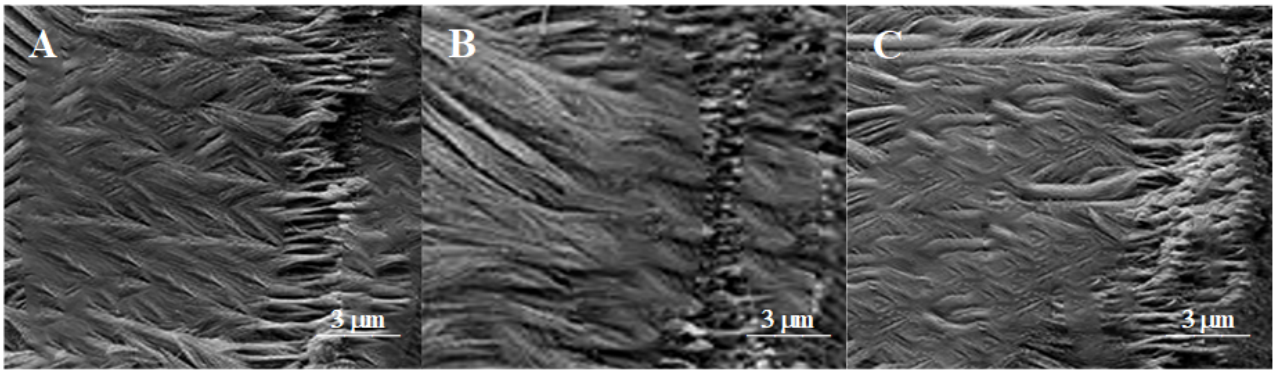

Fig. 2. The state of dental caries under a scan electron microscope. (Total magnification: ×1000) Note: (A) shows the experimental group, (B) shows the sodium fluoride group, and (C) shows the normal saline group. Scale bar: 3 μm.

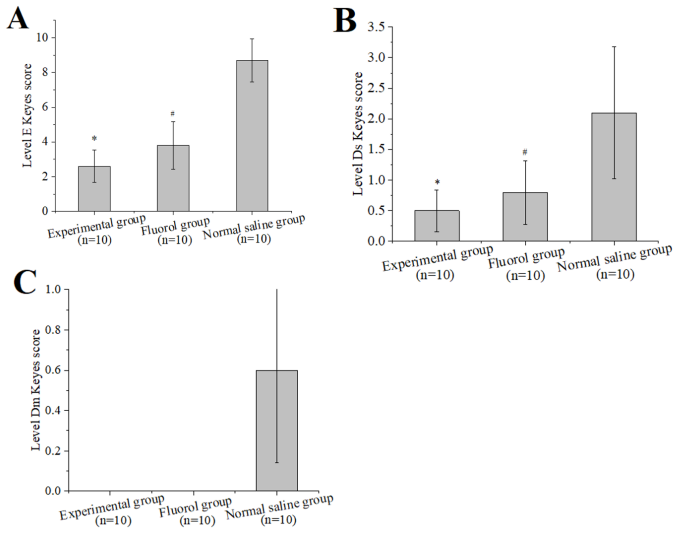

Fig. 3. Keyes scores for smooth surface caries in each group of rats. Note: (A) represents the enamel (E)-level, (B) represents the slight dentinal (Ds)-level, and (C) represents the extensive dentinal (Dx)-level. * indicates a statistically considerable difference ($p < 0.05$) between the experimental group and the saline group; # indicates a statistically considerable difference ($p < 0.05$) between the sodium fluoride group and the saline group.

Results

Comparison of Bacterial Colony Count in Molars of Rats in Each Group

Fig. 1 illustrates the progression of *Streptococcus mutans* colonies in the molars of rats across different experimental groups. The physiological saline group exhibited a significant increase in the number of *Streptococcus mutans* colonies over time ($p < 0.05$). Conversely, the sodium fluoride group and the experimental group demonstrated a notable decrease in the number of *Streptococcus mutans* colonies over time ($p < 0.05$).

The State of Dental Caries under Scan Electron Microscopic

Fig. 2 displays the status of caries under a scanning electron microscope. The experimental group exhibited characteristics similar to those observed after the application of restorative material. The restorative material successfully filled the defect on the tooth surface, resulting in a smoother surface with fewer carious lesions or cavities observed in the caries area. The sodium fluoride group showed the presence of carious lesions, while the saline group exhibited more pronounced carious lesions.

Keyes Score of the Smooth Surface of Dental Caries in Each Group

In Fig. 3, no caries damage was detected at the extensive dentinal (Dx)-level on the smooth surface of any tooth set. Both the experimental group and the sodium fluoride group exhibited no dental caries at the moderate dentin (Dm)-level. When compared to the saline group, the experimental group demonstrated a significant decrease in Keyes scores (E- and slight dentinal (Ds)-level) for smooth

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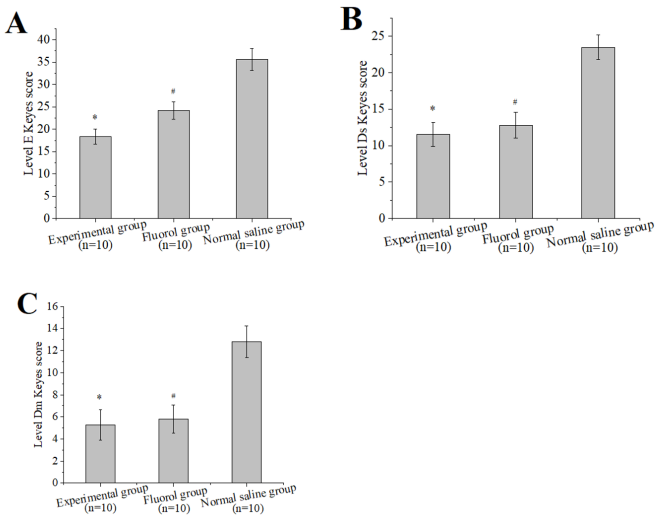

Fig. 4. Keyes scores for fissure surface caries in rats from each group. Note: (A) represents E-level, (B) represents Ds-level, and (C) represents Dx-level caries. * denotes a statistically considerable difference ($p < 0.05$) compared to the saline group for the experimental group; # denotes a statistically considerable difference ($p < 0.05$) compared to the saline group for the sodium fluoride group.

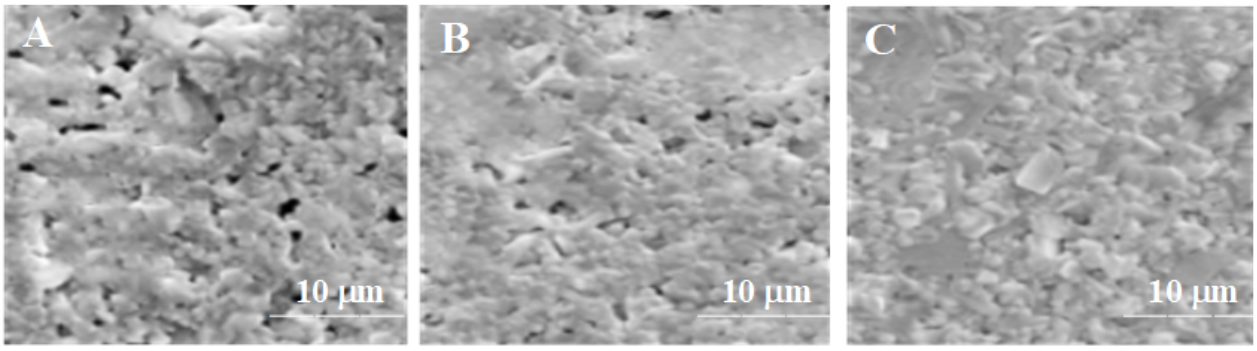

Fig. 5. Scan electron microscopy morphology of the tooth surface of rats in each group (Total magnification: ×1000). Note: (A) represents the saline group, (B) represents the sodium fluoride group, and (C) refers to the experimental group. Scale bar: 10 μm.

surface caries, showing statistical significance ($p < 0.05$). However, there was no statistically significant difference in Keyes scores for smooth surface caries between the experimental group and the sodium fluoride group ($p > 0.05$).

Keyes Score of Dental Caries Pits and Fissures in Each Group

In Fig. 4, the Keyes scores of enamel (E)-level, Ds-level, and Dx-level caries in both the experimental group and sodium fluoride groups were significantly lower than those in the saline group, demonstrating statistical significance ($p < 0.05$). Notably, there was no significant difference observed in the Keyes scores for Ds- and Dx-level caries between the experimental and sodium fluoride groups ($p > 0.05$).

Scan Electron Microscope Morphology of the Tooth Surface of Rats in Each Group

Then scanning electron microscopy images in Fig. 5 reveal differences in the enamel surface of rats among the three groups. In the saline group (Fig. 5A), the enamel surface appears rough, exhibiting numerous scratches and a high number of pits in the demineralized area. On the other hand, the sodium fluoride group (Fig. 5B) shows a significantly smoother enamel surface, with minimal demineralized areas and the presence of remineralization characteristics. The experimental group (Fig. 5C) exhibits a smooth enamel surface with a superficial demineralized area and a reduced number of pits, as compared to the saline group.

Discussion

Dental caries is a prevalent chronic disease that significantly impacts global oral health and quality of life. The

development of effective preventive measures and treatment methods is crucial for reducing caries incidence and improving oral health in individuals. HAP, an inorganic substance similar to tooth tissue composition, has emerged as a promising material in dentistry [19–21]. Investigating the role of HAP restorative coatings in caries prevention can provide further insights into their potential for tooth repair and protection. Research on HAP restorative coatings contributes to enhancing the effectiveness and durability of caries treatment. By filling and repairing carious lesions and cavities, HAP restorative coatings can effectively alleviate the severity and progression of dental caries, thereby prolonging the lifespan and stability of restorations [22]. Synthetic HAP has been increasingly utilized in dental applications, particularly in the replacement and restoration of dental caries. Babayevska *et al*. (2021) [23] investigated three different forms of HAP-DF composite materials, assessing their structural, morphological, elemental, and chemical properties. The study revealed that HAP nanoparticles remarkably improved the biological properties of dental caries and exhibited acceptable biocompatibility with selected human cells. Li *et al.* (2020) [24] successfully synthesized two self-assembling β-folded peptides, ID4 and ID8, can self-assemble into nanofibers, promoting the remineralization of initial enamel caries. However, the repair process using these techniques is relatively time-consuming. In this study, a restorative paste was prepared by mixing HAP powder with H_3PO_4/H_2O_2 reagents, using a paste-forming method. For comparison and evaluation, we established a sodium fluoride group, to assess the effectiveness of HAP restorative agents against traditional caries prevention treatments. Sodium fluoride is a widely used oral health substance, especially for the prevention of dental caries. Its primary mechanism of action involves strengthening the minerals on the tooth surface, inhibiting the growth and acid production of oral bacteria, and promoting the remineralization of damaged teeth.

Based on the experimental results, the following observations were found. After four weeks of treatment, the colony counts of *Streptococcus mutans* in the molar teeth of the saline group were higher compared to those in the experimental and sodium fluoride groups. Conversely, the colony counts of *Streptococcus mutans* in the molar teeth of the experimental group were lower than those in the sodium fluoride group. Additionally, the Keyes scores for smooth surface caries and fissure caries (E- and Ds-levels) in the experimental and sodium fluoride groups were lower than those in the saline group. These findings are consistent with the results reported by Shahmoradi *et al*. (2018) [25]. Several factors may explain these results. The experimental group was treated with HAP restorative coating, while the sodium fluoride group was treated with sodium fluoride solution. Both of these treatment methods may offer superior efficacy in caries prevention. The HAP restorative coating can fill carious cavities and lesions, thereby reducing bacterial colonization and the formation of acidic environments on the tooth surfaces. On the other hand, the sodium fluoride solution can enhance the acid resistance of teeth, reducing the formation of acid erosion caries. In contrast, the saline group did not receive any specific preventive treatment, leading to a higher incidence of caries formation and bacterial colonization. The higher Keyes scores for smooth surface caries and fissure caries (E- and Ds-levels) in the saline group may indicate a more severe acid erosion in the teeth. The treatment methods used in the experimental and sodium fluoride groups may have reduced the severity of acid erosion, resulting in lower caries scores and less severe caries lesions. However, there were no considerable differences in the Keyes scores for smooth surface caries between the experimental and sodium fluoride groups (E- and Ds-levels), nor in the Keyes scores for fissure caries between the two groups (Ds- and Dm-levels). This suggests that both treatment methods have similar effectiveness in reducing caries progression. Whether utilizing the HAP restorative coating or sodium fluoride solution, both methods can provide a certain level of protection and reduce the advancement of caries. Analysis using scanning electron microscopy (SEM) revealed that the tooth enamel surfaces of rats in the saline group appeared rough with scratches, displaying a higher incidence of demineralized areas and depressions. This may be attributed to the lack of specific treatment in the saline group, leading to increased acid erosion and enamel damage. In contrast, the tooth enamel surfaces of the rats in the sodium fluoride group were smoother, exhibiting fewer demineralized areas and visible signs of remineralization. This suggests that sodium fluoride solution can effectively enhance tooth acid resistance, thereby reducing the degree of acid erosion and protecting the integrity of the tooth enamel surface. In the experimental group, the tooth enamel surfaces of the rats displayed a smooth texture with significantly fewer and shallower demineralized areas. This indicates a strong inhibitory effect of the HAP coating on *Streptococcus mutans*, as well as an improvement in the surface structure of carious teeth.

The data analysis based on the Keyes scores revealed that the experimental group and the fluoride sodium group exhibited a substantial reduction in dental caries compared to the control group. Notably, the experimental group's dental caries Keyes scores were lower than those of the fluoride sodium group, although the difference was not statistically significant. This suggests that the experimental group, which utilized HAP repair coating, may have experienced a slight advantage in reducing tooth damage, as the coating potentially filled caries cavities and lesions. Consequently, this may have minimized bacterial colonization and acidogenic conditions on the occlusal surface. On the other hand, the sodium fluoride group employed a sodium fluoride solution to enhance acid resistance and mitigate the formation of acid erosion caries. The minimal variance ob-

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served between the two groups could be attributed to the similarity in their preventive effects against dental caries, resulting in similar levels of caries severity. Additionally, the application of HAP repair coatings in the experimental group likely contributed to the filling of caries cavities and lesions, thereby reducing surface defects. Simultaneously, the sodium fluoride solution enhanced acid resistance and inhibited the progression of acid erosion caries. Consequently, the similar effects of both treatment methods on dental caries in a negligible difference in Keyes scores. The strengths of this study include the use of an animal model, which enables better control of confounding factors and facilitates the observation of the effects of different treatment methods on dental caries. Moreover, multiple evaluation parameters were employed to comprehensively assess the pathological and morphological status of dental caries.

Although the present study evaluates the efficacy of HAP repair coatings within a four-week timeframe, the long-term stability and safety of these coatings remain critically important. To ensure their viability as long-term dental repair solutions, future research should prioritize long-term monitoring to determine the duration of treatment efficacy and identify any potential adverse reactions that may arise over time. This will involve the design of experiments with extended durations, which will enable the assessment of HAP coatings' durability in the oral environment and their impact on surrounding tissues. By doing so, we can ensure that HAP repair coatings are safe and effective for long-term use in dental repair applications. Additionally, this study has revealed a significant reduction in *Streptococcus mutans* counts following the application of HAP coatings. However, there is a lack of detailed discussion regarding the potential biological mechanisms of HAP coatings. Future research should delve into how HAP coatings affect the composition of oral microbiota and their molecular-level interactions with the enamel surface. By employing molecular biology techniques such as gene expression analysis and proteomics, the specific effects of HAP coatings on oral microbial balance can be elucidated, providing deeper insights into their mode of action in preventing dental caries. Furthermore, although studies based on rodent models provide preliminary data, the potential translation of research results into human oral health management has not been fully discussed. Given the differences between rodents and humans in oral environments and physiological responses, it is crucial to evaluate the relevance of research results for human patients. This includes exploring potential avenues to translate findings from animal models into human clinical applications, such as conducting preliminary human clinical trials, assessing treatment efficacy and safety, and fulfilling regulatory approval requirements. Collaborating with clinical researchers to design and implement clinical trials that assess the application of HAP coatings in human oral health will be a crucial step towards advancing research in this field. In summary, this study highlights the potential of HAP repair coatings in preventing dental caries. However, further research is necessary to understand their long-term effects, mechanisms of action, and feasibility in human application. Future work should focus on addressing these limitations and exploring pathways to translate this innovative treatment approach into practical clinical applications.

Conclusions

The results indicated that, in a rat model of dental caries induced by oral infection, both the experimental group treated with HAP restorative coating and the sodium fluoride group showed a significant reduction in bacterial colonies on dental caries lesions compared to the control group. Moreover, the reduction in dental caries bacterial colonies was more pronounced after treatment with HAP restorative coating, accompanied by a significant improvement in pathological conditions and a reduced level of damage. Although the difference in Keyes scores between the experimental group and the fluoride sodium group was not statistically significant, the Keyes scores for dental caries in the experimental group were lower than those in the fluoride sodium group. SEM observations revealed that the enamel surface in the experimental group appeared relatively smooth, with fewer demineralized areas and notable signs of remineralization. In contrast, the enamel surface in the fluoride sodium group appeared rougher, with visible scratches and more extensive demineralized areas. These findings suggest that HAP restorative coating has an inhibitory effect on bacterial colony formation and promotes dental caries repair in the treatment of oral infection-induced dental caries. This study offers promising new treatment approaches and references for caries prevention. However, further research is needed to evaluate the effectiveness and mechanisms of different preventive treatment methods, thus providing more reliable evidence for clinical practice.

Availability of Data and Materials

The data and materials used in this study were sourced from internal laboratory records, and some of the data were not made public due to confidentiality agreements and limitations of the research nature.

Author Contributions

SSS designed research plans, conducted experimental operations, and wrote and revised papers. QL was responsible for data collection and analysis. XB was responsible for the preparation of experimental materials and the preliminary processing of experimental data. CEQ was responsible for the care and handling of experimental animals. YL participated in experimental design and result discussion. LDB conducted literature review, analyzed data and final review

of the paper. All authors have been involved in revising it critically for important intellectual content. All authors gave final approval of the version to be published. All authors have participated sufficiently in the work to take public responsibility for appropriate portions of the content and agreed to be accountable for all aspects of the work in ensuring that questions related to its accuracy or integrity.

Ethics Approval and Consent to Participate

This study received approval from the Ethics Committee of the Affiliated Hospital of Chifeng University, adhering to established guidelines for the care (ethics approval number: 20203712), handling, and experimentation involving laboratory animals.

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Conflict of Interest

The authors declare no conflict of interest.

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