~~Experimental Study of~~ Photoactivated Disinfection ~~Procedure in the Treatment of~~for ~~for~~ Denture Stomatitis in Diabetic Rats

Xiao Zhang[#1], Zirui Zhao[#1], Ruiqi Zhang[1], Juan Liu[1], Zhijiao Guo[1], Qiaoyu Hu[1], Qing Liu[1], Na Liu[2]

[1] Hebei Key Laboratory of Stomatology, Hebei Clinical Research Center for Oral Diseases, School and Hospital of Stomatology, Hebei Medical University, Shijiazhuang, Hebei, PR China

[2] Department of Preventive Dentistry, School and Hospital of Stomatology, Hebei Medical University, Shijiazhuang, Hebei, PR China

Corresponding Authors:
Qing Liu
Yucai Street, Chang'an District, Shijiazhuang, Hebei, 050017, PR China
Email address: kqliuqing@sina.com
Na Liu
Yucai Street, Chang'an District, Shijiazhuang, Hebei, 050017, PR China
Email address: ynlqdx@sina.com

Abstract

Objective: To study the efficacy of using PAD™ ~~Plus-based~~Plus based photoactivated disinfection (PAD) for the treatment of denture stomatitis in diabetic rats by establishing a diabetic rat denture stomatitis model.

Methods: ~~To create t~~The diabetic rat denture stomatitis model involved~~,~~ the aleatory selectio<n of two-month-old male SD rats ~~were chosen~~ and randomly separated into four groups. Rats in the PAD groups had their palate and denture surfaces incubated with 1 mg/mL Toluidine Blue O (TBO) for 1 min each, followed by 1 min of exposure to 750 mW light-emitting diode (LED) light. The PAD-1 group received one radiation treatment; the PAD-2 group received three radiation treatments over the course of five days, one at a one-day interval. The NYS group received treatment for 5 days with a suspension of nystatin (NYS), 100,000 IU. The infection group did not get any treatment. In each group, inflammation score of palate~~,~~, tests for fungal load, histological evaluation, and immunohistochemical detection of IL-17 and TNF-~~α~~ α were ~~carried out~~conducted 1 and 7 days following the conclusion of treatment.

Results: At ~~1d~~ day one following treatment, the fungal load on the palate and dentures, as well as the mean optical density values of IL-17 and TNF-α, were all ~~substantially greater~~greater in the infection group than in the other three treatment groups (P<0.05). At ~~7d~~ the seventh day following treatment, the infection group of those were significantly higher than the PAD-2 group and NYS group (P<0.05). Importantly, ~~,~~there were no differences between the infection group and PAD-1 group nor in the~~,~~ PAD-2 group and NYS group ~~were not statistically significant~~ (P>0.05).

Conclusions: PAD can effectively reduce the fungal load and the expression of IL-17 and TNF-α in the palate and denture of diabetic DS rats. The efficacy of multiple light treatments is superior to that of single light treatments and ~~similar to~~like that of NYS.

Keywords: *Candida albicans*, Photoactivated disinfection, Diabetes mellitus, Denture stomatitis, IL-17, TNF-α

INTRODUCTION

Denture stomatitis (DS) is a common infection of the oral mucosa in denture wearers, and *Candida albicans* is the most significant etiological agent of DS (*Sugio et al., 2020*). Epidemiological studies have found that the incidence of DS in denture wearers ranges from 15% to 70% (*Gendreau & Loewy, 2011*). ~~DS~~ This stomatitis is commonly treated with antifungal drugs, but ~~the~~ their overuse ~~of these drugs~~ has led to an increased appearance of ~~in~~ drug-resistant *Candida albicans* strains. As an alternative, ~~Photodynamic~~ photodynamic therapy has emerged as a potential treatment option for DS, especially in patients with diabetes.

Diabetes mellitus (DM) is a common endocrine disorder that is escalating at an alarming way. According to the International Diabetes Federation, 642 million adults will have diabetes worldwide by 2040, up from 415 million in 2015 (*Zimmet et al., 2016*). According to some studies, ~~Patients~~ patients with DM are also more susceptible to opportunistic infections including oral candidiasis due to elevated serum glucose levels and decreased function of the cellular immune system. High blood glucose in saliva is one of the main risk factors for oral *Candida* infection in DM patients, with over 77% of DM patients suffering from oral candidiasis (*Soysa et al., 2006*). Studies have shown that DM patients have a higher prevalence of *Candida albicans* carriage compared to normal individuals, and that their oral mucosa is more susceptible to fungal infections. The incidence of DS notably increases in patients with DM following the repair of removable dentures, which further complicates the clinical management of DS ~~After removable denture repair, the incidence of DS in DM patients is also significantly increased, increasing the difficulty of clinical treatment of DS~~(*Javed et al., 2009*). *SM et al.* (*2014*) investigated the sensitivity of antifungal drugs to *Candida* in diabetic mice and found that high blood glucose levels reduce the activity of antifungal drugs. The presence of high blood glucose factors undoubtedly brings new challenges to the treatment of DS and finding an effective clinical treatment method that does not produce drug resistance is particularly urgent.

PAD is a novel therapy that selectively kills diseased cells or tissues through a photodynamic reaction generated by the interaction of light, photosensitizers (PS) and oxygen, without damaging other normal tissues. Its main advantage is that microorganisms are less likely to develop resistance to reactive oxygen species (ROS)(*Abdelkarim-Elafifi et al., 2021*). In recent years, the antimicrobial effect of PAD on *Candida albicans* has been confirmed, and scholars have started to focus on the study of PAD in the treatment of DS. ~~Most existing~~Most clinical studies have confirmed that PAD can effectively treat DS, but the treatment duration is ~~generally long~~long and requires multiple sessions. For example, *Mima*

Comentado [EO1]: Why is the most significant? What makes candida stomatitis more significant than those caused by other infectious agents? Please explain.

Comentado [EO2]: Which are the factors that influence this broad range?

Comentado [EO3]: I believe the introduction of phototherapy as a treatment in the paper is prematurely and contextually misplaced. It would be far more beneficial for the reader if the context provided included the advantages and potential of the technique. This should be concise, yet comprehensive enough to justify the abrupt introduction of this term in the context of treating this infection.

Comentado [EO4]: To support this, I would recommend to insert another reference, for example: Gianchandani, R., Esfandiari, N., Ang, L., Iyengar, J., Knotts, S., Choksi, P., & Pop-Busui, R. (2020). Managing Hyperglycemia in the COVID-19 Inflammatory Storm. *Diabetes*, 69, 2048 - 2053. https://doi.org/10.2337/dbi20-0022.

Comentado [EO5]: I also suggest to introduce a paper supporting this observation, for example: Khanna, M., Challa, S., Kabeil, A., Inyang, B., Gondal, F., Abah, G., Dhandapani, M., Manne, M., & Mohammed, L. (2021). Risk of Mucormycosis in Diabetes Mellitus: A Systematic Review. *Cureus*, 13. https://doi.org/10.7759/cureus.18827.

Comentado [EO6]: Please, insert here a study that supports this statement.

et al. (*2012*) incubated with 500 mg/L Photogem for 30 min, followed by 20 min of light irradiation. *Alves et al.* (*2020*) incubated with 200 mg/L Photodithazine for 20 min and irradiated for 4 min. Both studies ~~are~~ were ~~illuminated~~ conducted ~~3~~three times per week, with a total of ~~6~~six times per 15 days. This is a lengthy treatment duration, and prolonged mouth opening during oral treatment may lead to temporomandibular joint disorders, excessive saliva secretion, etc. The treatment effect will be ~~greatly affected~~affected, so reducing the oral operation time is crucial.

Fortunately, we have learned about the PAD™ Plus-based PAD technology that can effectively eliminate *Candida albicans* within ~~a short period~~a brief period. The technology uses a complementary pharmaceutical grade 1 mg/mL TBO solution and a 635 nm red LED with an output power of 500 mW or 750 mW and ~~the~~ an irradiation time of 1 min or 2 min. The TBO solution is activated at this wavelength to produce ROS, which selectively kills microorganisms. In *vitro* studies have found that increasing the concentration of photosensitizers enhances the inhibitory effect on biofilms (*Pinto et al., 2018*). Previous studies showed that 1 mg/mL TBO for 1 min and 750 mW LED irradiation for 1 min had a good inactivation effect on *Candida albicans* on the dorsal tongue of mice (*Gu et al., 2022*). Zhang et al. (2023) found that incubation with 1 mg/mL TBO for 1 min, followed by 1 min of 750 mW LED irradiation or 2 min of 500 mW LED irradiation, can inactivate over 99% of *Candida* in the mature mixed biofilms. Therefore, in this study, we selected 1mg/mL TBO solution and 750 mW LED red light for 1 min to explore the therapeutic effect of ~~different times~~various times of light on DS in diabetes rats, in order to provide a rapid and efficient treatment method for clinical treatment of DS.

MATERIALS AND METHODS

Preparation of fungal suspension

The *Candida albicans* SC5314 was provided by Shijiazhuang Hera Biotechnology Co., Ltd. The *Candida albicans* strain stored at -80℃ was inoculated onto CHROMagar™ Candida chromogenic medium (Comagal microbial technology Co., Shanghai, China) and incubated at 37℃ for 24 hours in a constant temperature incubator. After growth, a single colony was ~~then~~ selected and re-inoculated onto a new Candida chromogenic medium, incubated at 37 ℃ for another 24 hours. The activated single colony was ~~then selected and~~ inoculated into 20 mL of yeast extract-peptone-dextrose medium broth medium for amplification at 37℃ with shaking at 150 rpm overnight. The resulting fungal suspension was ~~aspirated into a centrifuge tube,~~ centrifuged at 4000 rpm for 15 min in a high-speed centrifuge, and the supernatant was discarded. The fungal cells were washed with 10 mL of PBS solution, the above centrifugation and washing steps were repeated three times before discarding the supernatant to collect the fungal pellets. The pellets were stored at 4℃ until use.

Comentado [EO7]: I would recommend to insert this citation to support the finding related to ROS production: Baltazar, L., Soares, B., Carneiro, H., Ávila, T., Gouveia, L., Souza, D., Ferreira, M., Pinotti, M., Santos, D., & Cisalpino, P. (2013). Photodynamic inhibition of Trichophyton rubrum: in vitro activity and the role of oxidative and nitrosative bursts in fungal death.. *The Journal of antimicrobial chemotherapy*, 68 2, 354-61 . https://doi.org/10.1093/jac/dks414.

Comentado [EO8]: Could you please specify the sources of the reagents employed in the YPD preparation? The origin of peptone, in particular, can significantly influence experimental outcomes and it's important to have this information for accurate interpretation of the results.

Experimental animals and establishment of diabetes model in rats (*Deeds et al., 2011; King, 2012*)

Calculated based on the animal attrition rate of about 10% in the pre-experiment, forty male SD rats, 2 months old, weighing from 300-350g, were purchased from Beijing Huafukang Biological Technology Co., Ltd. (animal quality certificate number: 110322220101519632). The rats were acclimatized for 1 week and maintained under constant temperature and humidity with free access to food and water. This study was approved by the Ethics Committee of the Hospital of Stomatology, Hebei Medical University (Approval NO.: [2020]016).

A total of 40 rats were fasted for 12 hours and then injected intraperitoneally with 60 mg/kg of 1% streptozotocin to induce diabetes. On the day of streptozotocin injection, the rats were ~~given~~ feed with 5% sterile glucose water to prevent hypoglycemia, and normal sterile water was provided on the following day. After 72 hours, rats with a fasting blood glucose concentration >16.7 mmol/L were considered to have successfully developed diabetes and were included in the experiment, while one rat that did not meet this criterion was excluded.

Comentado [EO9]: Perhaps you should mention here the number of subjects that accomplished the criteria.

Denture fabrication (*Yano et al., 2016*)

The alginate impression material was evenly placed on a tongue depressor, and then placed in the rat mouth to obtain an impression of the palate (Fig. 1A). A corresponding gypsum model was made according to the impression (Fig. 1B). The denture was then prepared using light-cured acrylic resin on the gypsum model (Fig.1C) to approximately 3 mm thickness and covered the entire hard palate area. After polishing, it was placed in distilled water at 37℃ for 48 hours to release residual monomers. The denture was then immersed in sterile distilled water and microwaved at 650W for 3 min to achieve sterilization.

Denture seeding (*Lie Tobouti et al., 2016*)

On the day of denture ligation, the prepared fungal suspension was diluted in PBS and counted using a hemocytometer to a concentration of 1×10^7 CFU/mL in ~~RPMI~~ RPMI-1640 medium for denture seeding. To allow *Candida albicans* to adhere to the denture tissue surface, the denture was placed in a six-well tissue culture plate, and each well contained 1×10^7 CFU/mL of *Candida albicans* suspension in ~~RPMI~~ RPMI-1640 medium. The six-well plate was placed in 37℃ water bath oscillation incubator and shaken at 75 rpm for 90 min. The denture was then gently immersed in 2 mL of PBS to remove any non-adherent fungal cells.

Comentado [EO10]: Please include details about the quantity of suspension added to each well, or alternatively, provide the total fungal load used for denture colonization. This information is crucial for understanding the experimental setup and replicating the study accurately

Denture ligation

Rats were anesthetized by intraperitoneal injection of 0.6% sodium pentobarbital 40 mg/kg. Two 5 cm long, 0.2 mm diameter stainless steel ligatures were threaded between the first and second molars on both sides of the maxilla, and through the holes on both sides of the

denture. The excess wire at the end was cut, and the tip was covered with self-curing resin to protect the oral soft tissues of the rat. A bacterial suspension with a concentration of 1×10^9 CFU/mL was applied to the rat's palate after denture ligation. Preceding modeling experiments have shown that a stable denture stomatitis model in diabetic rats can be established after 3 weeks of ligation and denture seeding.

Comentado [EO11]: Shouldn't this section refer to 'fungal' instead of 'bacterial'? Additionally, it's important to specify the administered volume for clarity and precision in the methodology.

PAD treatment methods

A total of 36 rats were selected as molded rats, and the random number table method was divided into 4 groups (n=9 per group). For the PAD-1 group, 1 mg/mL TBO solution (Denfotex, UK) was applied to the palate and denture tissue surface with a small brush, incubated for 1 min, and then irradiated with 750 mW output power for 1 min (PAD™ Plus instrument, Denfotex, UK; model: DX9001). The light source was systematically maneuvered ~~was moved~~ to ensure that the entire palate and denture surface were ~~covered~~irradiated. This was done once a day for one day. For the PAD-2 group, the same treatment as PAD-1 group was given, but with an interval of 1 day for irradiation, and a total of ~~3~~three times were irradiated within 5 days. The NYS group was treated with 100,000 IU of nystatin suspension applied to the palate and denture once a day for five consecutive days, while the infection group was not treated.

Efficacy observation and euthanasia

The rats in each group were anesthetized by intraperitoneal injection of 0.6% sodium pentobarbital ~~sodium~~ at 40 mg/kg 1d and 7d after the end of the treatment. The samples underwent two assessments: first, scoring for the extent of palatal inflammation, and second, measuring the fungal burden on both the palate and the denture tissue surface. ~~, and were subjected to the scoring of the degree of inflammation of the palate, and the determination of the fungal load of the palate and the denture tissue surface, respectively.~~ Three rats in each group were randomly executed 1d after the end of treatment (death by inhalation anesthesia with excessive isoflurane), and all remaining rats were executed 7d after the end of treatment~~, and~~. ~~t~~The palate tissues were taken for histopathological examination and immunohistochemistry to detect the changes of IL-17 (Boorsen Biotechnology Co., Beijing, China) and TNF-α (Bowan Biotechnology Co., Shanghai, China). Since preceding experiments have proven that the stable period of the model occurs at 3–6 weeks after denture seeding, the infection group was evaluated at the same time points of PAD-1 group and PAD-2 group after treatment (at 1 day and 7 days respectively), with the average data of the two time points compared to eliminate bias caused by time difference. The effectiveness of PAD therapy for diabetic rat denture stomatitis was ~~then~~ evaluated based on the results obtained from these evaluations.

Palate mucosal inflammation score. The Newton's method was used to visually evaluate the palatal tissues, and scores were assigned based on the severity of inflammation

(Johnson et al., 2012). 0~~,~~: no inflammation; 1~~,~~: punctate erythema; 2~~,~~: diffuse erythema and edema; 3~~,~~: diffuse erythema/edema and papillary hyperplasia.

Palatal and denture tissue surface fungal ~~load~~ burden measurement. The palate and denture of each group of rats were swabbed with sterile cotton for 1 min. The cotton swab was then placed into a centrifuge tube containing 1 mL of saline and shaken for 1 min. A 10 μL aliquot of each dilution was spread onto BIGGY agar culture medium and cultured at 25 ℃ for 48 hours. The ~~bacterial~~ fungal colony count was measured in CFU/ml and analyzed statistically by taking the logarithm of the CFU number.

Histopathological examination. Routine hematoxylin and eosin (HE) staining, periodic acid-Schiff (PAS) staining and immunohistochemical (IHC) staining were performed, and microscopic observation and image collection were conducted.

Comentado [EOZ12]: In this section, it would be beneficial to provide a brief yet detailed description of how the PAS and IHC staining procedures were performed. Including specific details will aid in accurately replicating your findings and understanding your methodology more clearly.

Statistical analysis

Data analysis was performed using SPSS25.0 software (IBM Corp., USA). The measurement data was tested for normality and homogeneity of variance tests. If normality and homogeneity of variance were met, two-factor ANOVA was used and group comparisons were performed using t Student~~-~~ tests. Otherwise, the Friedman rank sum test was used and group comparisons were performed using the K~~-~~ruskal~~-~~Wallis test. As the grade data (the palate mucosal inflammation score of the rats) used a constituent ratio, it was described and analyzed with the Friedman rank sum test. $P < 0.05$ was set as the level considered to be statistically significant.

RESULTS

Palate mucosal inflammation score

One day after treatment, ~~The~~ the infection group displayed apparent symptoms of swelling and redness of the palate ~~1d after treatment,~~ and the inflammation score was primarily 2 (Figure 2A). In the three treatment groups, there was less inflammation in the palate, with scores typically between 0 and 1 (Figure 2B-D~~,~~). The infection group's palate inflammation persisted ~~to get~~and ~~-~~worsened at ~~-~~day 7~~d after treatment~~, with inflammation scores typically at 2–3 points (Figure 2E). Partial redness and edema of the palate were ~~seen~~ also observed in the PAD-1 group, with scores typically ranging from 1 to 2 points (Figure 2F). The PAD-2 group and NYS group, with scores primarily at 0–1 point, did not exhibit any discernible redness and swelling in the palate (Figure 2 G and H)). At 1d following treatment, pairwise comparisons revealed that the infection group's inflammation scores were considerably ~~greater~~ higher than those of the three treatment groups ($P<0.05$). The inflammatory score in the infection group was noticeably greater than that in the PAD-2 group 7d after treatment ($P<0.05$). The inflammatory scores showed no significant difference between the infection

Comentado [EOZ13]: Was there a difference in the scores among the treatments on both day 1 and day 7?

group, PAD-1 group, and NYS group (P>0.05) as illustrated in Figure 3. However, it is noteworthy that the PAD-2 group demonstrated a statistically significant reduction in palatal inflammation with a *P* value of 0.024, indicating its efficacy is potentially greater than that of the NYS group. These findings suggest that only the PAD-2 treatment, can effectively reduce inflammation in this context.~~The inflammatory score between the infection group, PAD-1 group, and NYS group did not differ significantly (*P*>0.05) (Figure 3). These results show that PAD can reduce the inflammation of the palate in rats, although the PAD-1 group showed recurring symptoms, but the efficacy of the PAD-2 group was similarly to that of the NYS group.~~

Fungal ~~load~~ burden analysis

The fungal ~~load~~ burden in the palate and dentures of PAD-1 group was significantly higher at 7d after treatment than at 1d after treatment (*P*<0.05). However, there were no significant differences in the palate and denture fungal load between the infection group, PAD-2 group, and NYS group at either 1d or 7d after treatment (*P*>0.05) (Figure 4).

As illustrated in Figure 5A, w~~W~~hen comparing pairwise, the fungal load in the palate of the three treatment groups was significantly lower than that in the infection group 1d after treatment, with statistical significance (*P*<0.05~~).~~). There were no significant differences in the palate fungal load between the three treatment groups (*P*>0.05). On the 7d after treatment, the fungal load in the palate of the infection group and PAD-1 group was significantly higher than that of the PAD-2 group and NYS group (Figure 5 B, *P*<0.05). There was no statistically significant difference between the PAD-1 group and infection group, as well as between the PAD-2 and NYS groups (*P*>0.05) (Figure 5B). These results suggest that, when used efficiently, ~~-~~PAD can kill *Candida albicans*, ~~and the efficacy of the PAD-2 group was better than that of the PAD-1 group, and was~~ in a grade similar to that of the ~~NYS group~~nystatin.

When comparing pairwise, the denture fungal ~~load~~ burden in the infection group was significantly higher than that in the three treatment groups 1d after treatment, with statistical significance (Figure 4B and 6, *P*<0.05). There were no significant differences in the denture fungal ~~load~~ colonization between the three treatment groups (*P*>0.05). However, 7d after treatment, the denture fungal load in the infection group was significantly higher than that in the PAD-2 group and NYS group (Figure 6B, *P*<0.05). ~~And~~ Interestingly, the denture fungal burden~~load~~ in the PAD-1 group was also significantly higher between day 1 and 7 (Figure 4B) than that in the PAD-2 group, with significant differences (Figure 6B, *P*<0.05). However, there were no significant differences in the denture fungal load between the PAD-1 group and infection group, the PAD-2 group and NYS group, as well as between the PAD-1 group and NYS group (*P*>0.05) (Figure 6B).

Palate tissue histology

As can be observed in the HE staining of the tissue in Figure 7, 1d after colonization, ~~treatment, HE staining showed that~~ the infection group had notable thickening of the epithelial layer with papillary projections (7A), while the epithelial layer structure appeared ~~relatively~~ more homogenous ~~normal~~ in the three treatment groups (Figure 7B-D). ~~There were no significant differences in epithelial structure among the treatment groups.~~ In contrast, 7 days after treatment, the epithelial tissue in the infection group showed significant and abnormal proliferation, while partial proliferation was observed in the epithelium of the PAD-1 group (Figure 7E and F). The epithelial structure appeared ~~relativ~~more ~~ely normal~~normal in the PAD-2 group and NYS group, and there were no significant differences in epithelial structure among these treatment groups (Figure 7G and H).

The presence of the fungal colonization was analyzed with a PAS staining of the mucosa. 1d after treatment, PAS staining showed that a number of *Candida albicans* yeast cells were adhered to the tissue surface in the infection group, and some hyphae were observed to invade the superficial epithelium. The number of *Candida albicans* in the three treatment groups was ~~significantly~~ less evident than that in the infection group, and occasional *Candida albicans* yeast cells were observed to adhere to the mucosal surface but without hyphae invasion (Figure 8 A-D). There were no significant differences in fungal load among the treatment groups. 7d after treatment, a large number of *Candida albicans* yeast cells adhered to the mucosal surface in the infection group, and hyphae were visible invading the superficial part of the epithelium (Figure 8E). The PAD-1 group showed a intermediate number of *Candida albicans* yeast cells adhered to the mucosal surface, and partial hyphae invasion ~~in~~ of the epithelium, but less than that showed of the infection group (Figure 8F). Occasionally, a few *Candida albicans* yeast cells were observed on the mucosal surface in the PAD-2 group and NYS group (Figure 8G and H)~~). These results~~ supporting the previous findings.

The Effect of PAD on IL-17 and TNF-α Expression Levels in the Palate Mucosa of Diabetic DS Rats

IL-17 is a secretory protein that can be detected through immunohistochemistry as ~~expressed in~~ a brownish-~~-~~yellow pattern in the cytoplasm and intercellular space of various layers of epithelial cells in normal tissues. 1d after treatment, the infection group showed strong positive expression in various layers of epithelial cells, stroma, and vascular endothelial cells (Figure 9A). The treatment groups were ~~mainly weakly~~weakly positive in epithelial cells, stroma, and vascular endothelial cells, with lighter staining compared to the infection group (Figure 9 B-D). After 7d of treatment, the infection group and PAD-1 group showed a strong positive expression, ~~mainly in~~in epithelial cells, stroma, and vascular endothelial cells (Figure 9E and F). In comparison, ~~T~~the PAD-2 ~~group~~group, and NYS group showed a weaker ~~positive~~ expression pattern, ~~mainly in~~in epithelial cells, stroma, and

vascular endothelial cells, with lighter staining compared to the infection group and PAD-1 group (Figure 9G and H).

On the 1d after treatment, the average optical density value of the infection group was significantly higher than that of the treatment groups (Table 1, a super index $P<0.05$). The average optical density value of PAD-1 group was also slightly higher than that of PAD-2 group and NYS group ($P<0.05$). There was no significant difference in the optical density value between the PAD-2 group and NYS group (Table 1, b super index $P>0.05$). At 7d after treatment, the average optical density values of the infection group and PAD-1 group were significantly higher than those of the PAD-2 group and NYS group ($P<0.05$). There was no significant difference in the optical density value between the infection group and PAD-1 group, the PAD-2 group and NYS group ($P>0.05$) (Table 1).

TNF-α can also be expressed in normal tissues, mainly in the cytoplasm of cells throughout the epithelial ~~layer, and~~layer and can be seen as yellow-stained particles in the cytoplasm with immunohistochemistry. Strong positive expression of TNF-α was observed in the epithelial cells and vascular endothelial cells of the infection group. 1d after treatment, weak positive expression of TNF-α was ~~observed~~ detected ~~mainly in~~in the epithelial ~~cells~~ and vascular endothelial cells of the treatment groups, with lighter staining compared to the infection group. 7d after treatment, strong positive expression of TNF-α was observed in the surface layer and basal layer of the epithelium and vascular endothelial cells of the PAD-1 group, and positive expression was observed in the granular layer and spinous layer. Weak positive expression of TNF-α was ~~mainly observed~~observed in the epithelial ~~cells~~ and vascular endothelial cells of the PAD-2 group and NYS group, with lighter staining compared to the infection group and PAD-1 group (Figure 10).

When comparing pairwise, the average optical density value in the infection group was significantly higher than that in the treatment groups 1d after treatment ($P<0.05$). On the other hand, ~~T~~there was no significant difference in the optical density value between the treatment groups ($P>0.05$). 7d after treatment, the average optical density values in the infection group and PAD-1 group were significantly higher than those in the PAD-2 group and NYS group ($P<0.05$). While there was no significant difference in the optical density value between the infection group and PAD-1 group, as well as between the PAD-2 group and NYS group ($P>0.05$) (Table 2).

DISCUSSION

PAD is a non-invasive treatment method that was initially developed in dermatology and later applied in cancer treatment. In recent years, it has been ~~gradually applied~~ adopted in the treatment of oral mucosal diseases. Its mechanism of action is based on the interaction between a specific wavelength light source and PS in the presence of oxygen, inducing

Comentado [EOZ14]: Which wavelength and program was used to obtain this values?

Comentado [EOZ15]: It seems that the images selected for comparison among the different treatment groups in the immunohistochemistry assays are not entirely comparable. They display different areas of the dermis and epidermis of the chosen mucosal tissue. For a more accurate comparison, it would be beneficial to use images that are more uniform in terms of the anatomical regions they represent. This would ensure a clearer and more consistent comparison across the groups.

Comentado [EOZ16]: Insert a reference.

specific cell damage. PS absorbs photons from a specific wavelength light source, causing the energy to transition from the lower ground state to the higher excited singlet state. The excited singlet state may decay over time with laser irradiation or transition to the triplet state. This excited triplet state can undergo two reactions: In Type I reaction, electrons or hydrogen molecules are stripped from the substrate to generate highly active free radicals, which then react with endogenous oxygen molecules to produce ROS such as hydrogen peroxide, hydroxyl radicals, and superoxide~~. This reaction induces irreversible~~ that cause massive cellular damage. In Type II reaction, the PS reaches the triplet excited state and reacts with oxygen molecules that exist in the target cells such as gram-negative and gram-positive bacteria~~ cells~~, producing highly reactive oxygen or singlet oxygen molecules. Oxidative damage affects the target cells' plasma membrane, including proteins, lipids, and DNA, leading to cell death without affecting host cell activity (*Abdelkarim-Elafifi et al., 2021*). Among them, t~~T~~ype II reaction is commonly used for anti-infective treatment (*Konopka & Goslinski, 2007*).

Recent years have seen a significant shift in the PAD on DS research, with a greater emphasis on clinical trials and fewer fundamental investigations. Since clinical research is not convenient to carry out relevant studies on pathology and inflammatory factors, this study uses PAD^{TM} Plus-based PAD technology to treatment of DS in diabetic rats. The results of this study demonstrated that PAD ~~can~~could alleviate palatal inflammation in rats, and all treatment groups showed good ~~bactericidal~~ fungicidal effects against *Candida albicans* 1d post-treatment. The PAD-2 group had the lowest fungal ~~load~~ burden when it came to the palatal and denture samples from the treatment groups compared to the infection group. As a result, this experiment's outcomes for reducing inflammation and sterilizing the palate are comparable to those of some earlier clinical research (Afroozi et al., 2019; Alrabiah et al., 2019; Mima et al., 2011; de Senna et al., 2018).

Due to prior research indicating the possibility of disease recurrence during the follow-up phase after DS treatment, this study continued observation until 7 days post-treatment (Alves et al., 2020; Mima et al., 2011). ~~At 7d~~After 7 days of ~~post~~ treatment, the palatal and denture fungal ~~loads~~ burden in the PAD-1 group showed no statistically significant difference compared to the infection group, suggesting the onset of DS recurrence. However, both the PAD-2 ~~group~~group, and NYS group exhibited significantly lower fungal loads on the palate and dentures at 7d post-treatment, although slightly higher compared to 1d post-treatment. This observation might be linked to the acrylic resin composition of the dentures, which can function as a reservoir for microorganisms, thereby posing a risk of patient re-infection. Furthermore, replacing old dentures is often crucial for the full resolution of DS, particularly when the dentures are significantly aged. Regular renewal of dentures, therefore, plays a vital role in the effective management and prevention of this infection. ~~This observation may be attributed to the surface of acrylic resin dentures serving as a reservoir for~~

~~microorganisms, and is therefore, a potential source of re-infection of patients. Additionally, replacement of dentures is also necessary for complete resolution of DS, especially when dentures are very old.~~

There was no statistically significant fungal ~~load~~ burden on the palate and denture in the PAD-2 group and the NYS group at 1d and 7d post-treatment, indicating that the efficacy of multiple photodynamic therapy was similar to that of traditional treatment with ~~NYS~~nystatin, which is in line with the experimental results of Scwingel et al.（2012）.

Significantly, PAD stands out for its ~~requires a~~ shorter treatment duration, ~~significantly~~ markedly ~~reducing~~ decreasing both the ~~treatment~~ time and frequency ~~for~~ of treatment for patients, ~~enhancing~~ thereby offering greater convenience. ~~Importantly~~Additionally, PAD's low likelihood of developing ~~shows low potential for~~ drug ~~resistance,~~resistance, coupled with its ~~demonstrates~~ biocompatibility~~, and has fewer~~ and minimal side effects, position it as a highly favorable option. Thus, PAD ~~appears to be~~emerges as a promising approach for DS treatment.

Currently, few studies have documented the ~~research on the~~ immunological response during ~~y of~~ DS ~~is relatively rare~~. Th17 cells secrete pro-inflammatory cytokine IL-17. In the presence of local inflammation, IL-17 binds to the receptor expressed on the mucosal epithelial cells in the oral cavity, releasing relevant chemokines and stimulating the massive secretion of inflammatory cytokines, promoting or exacerbating the inflammatory response(*Lee et al., 2015*). Studies have shown that IL-17 from the same cell can not only induce neutrophils to eliminate pathogens to play a protective role, but also induce excessive inflammatory reactions ~~to damage~~that damage the tissue ~~s~~(*Matsuzaki G, 2018*). IL-17 and TNF-α play a synergistic role in the pathogenesis of diseases. IL-17 can induce macrophages to secrete IL-1β and TNF-α(*McGeachy et al., 2019*). In addition to local lesions, IL-17A can induce inflammation alone or in combination with TNF-α, causing immune cell gathering, fibrosis, and inducing systemic diseases(*Robert & Miossec, 2017*).

In recent years, the identification of genetic defects in the Th17/IL-17 axis in both, ~~genetic defects in~~ mice and humans, have revealed the importance of this pathway in controlling white Candida infection. ~~Research shows that p~~Patients with ~~potential~~ specific defects in IL-17-~~related~~ immunity, such as mutations affecting IL-17 production or receptor function, have been shown to be more susceptible ~~defects are more prone~~ to chronic cutaneous or mucosal candidiasis. Before identifying Th17 cells, the immune response mediated by IL-12 and Th1 cells was considered to play a major protective role in mucosal candidiasis (*Conti & Gaffen, 2010*). Schönherr *et al.* (*2017*) constructed a mouse oral candidiasis model and found that while the neutrophil recruitment and inflammatory response ~~caused~~ triggered by IL-17 ~~differed~~ varied ~~significantly~~ markedly ~~depending on~~across different ~~the~~ *Candida* species, ~~but~~ the necessity of IL-17 ~~requirement~~ for establishing ~~fungal~~ mucosal immunity ~~was~~ against fungal infections remained consistent for ~~the same~~all the fungal species

Comentado [EO17]: I recommend adding a transition in this section to more clearly articulate the rationale behind focusing on the production of both cytokines in this study, particularly within the context of diabetes.

Comentado [EO18]: Which diseases? Please specify.

Comentado [EO19]: The claim that IL-17A 'induces systemic diseases' appears quite broad. Could you please elaborate on what specific aspects or roles of IL-17A you are referring to in this context? What particular systemic diseases or mechanisms are you associating with IL-17A's activity?

Comentado [EO20]: In this section, when you mention the 'white' phase of the fungus, are you referring to its phenotypic white phase? Is this related to the well-known white/opaque switching phenomenon? If this term is not directly related to that specific phenomenon, I suggest omitting 'white' to avoid potential confusion.

examined. *Conti et al.* (*2009*) also showed that Th17-deficient mice were ~~highly sensitive~~extremely sensitive to oral pharyngeal candidiasis. Studies have found that IL-17A and IL-17RA knockout mice are ~~more~~ prone to *Candida* infection than wild-type mice in systemic *Candida* infections(*Saijo et al., 2010*).

In summary, this study ~~used~~ demonstrated the conditions required for the treatment of DS in a diabetic rat model. Specifically, we propose using 1 mg/mL TBO solution incubation for 1 min, combined with 750 mW output power LED light source for 1 min ~~to treat DS in diabetic rats~~. Our study contributes to the demonstrate that ~~T~~this technology ~~significantly~~ reduce~~d~~s the operation time compared to previous studies, making it more practical and convenient for oral therapy. The experimental results showed that using PAD to treat DS can ~~greatly reduce~~reduce the ~~load~~ burden of *Candida albicans* in the palate and dentures of rats, ~~improve~~ decrease the palate inflammatory symptoms, and reduce the expression of IL-17 and TNF-α in the palate tissues of diabetic rats with DS. ~~The~~ We found that ~~therapeutic effect of~~ multiple light treatments ~~is~~are better than ~~that of~~ a single light treatment, and no adverse reactions were observed in terms of safety. However, there are some limitations in this study. DM ~~is~~ was not controlled during the experiment, whether the existence of DM will have an impact on the treatment effect and long-term prognosis has not been deeply explored. Additionally, a small number of *Candida albicans* remained after the treatment, suggesting that future experiments could consider controlling for DM, increasing the number of ~~irradiation~~irradiation, or combining with antifungal drugs to develop a convenient and effective treatment protocol.

ADDITIONAL INFORMATION AND DECLARATIONS

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Declaration of interest statement

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Ethics approval

The use of animals in this study was approved by the Ethics Committee of the Hospital of Stomatology, Hebei Medical University, approval number: [2020]016.

Data Availability

Comentado [EO21]: While the discussion in the paper highlights the significance of the Th17/IL-17 axis, the authors do not delve into their findings in this area. Specifically, they do not propose a hypothesis to explain the observed differences in the immunohistochemical assays related to treatments with nystatin or phototherapy. Discussing this would be particularly crucial, especially in the context of the response in a diabetic rat model. It would also be beneficial if the authors could address the production of TNF-alpha and the observed differences, and further elaborate on their discussion regarding the production of both cytokines.

The following information was supplied regarding data availability: The raw measurements are available in the Supplemental Files.

Author Contributions

Xiao Zhang: Animal experiments, data statistics, writing manuscripts.

Juan Liu, Ruiqi Zhang, Zirui Zhao, Zhijiao Guo, Qiaoyu Hu: Animal experiments.

Na Liu: Experiment design, review manuscript.

Qing Liu: Experiment design, review and edit manuscript.

References

Abdelkarim-Elafifi, H., Parada-Avendaño, I., and Arnabat-Dominguez, J. 2021. Photodynamic Therapy in Endodontics: A Helpful Tool to Combat Antibiotic Resistance? A Literature Review. Antibiotics 10:1106. 10.3390/antibiotics10091106

Afroozi, B., Zomorodian, K., Lavaee, F., Zare Shahrabadi, Z., and Mardani, M. 2019. Comparison of the efficacy of indocyanine green-mediated photodynamic therapy and nystatin therapy in treatment of denture stomatitis. Photodiagnosis and Photodynamic Therapy 27:193-197. 10.1016/j.pdpdt.2019.06.005

Alrabiah, M., Alsahhaf, A., Alofi, R.S., Al-Aali, K.A., Abduljabbar, T., and Vohra, F. 2019. Efficacy of photodynamic therapy versus local nystatin in the treatment of denture stomatitis: A randomized clinical study. Photodiagnosis and Photodynamic Therapy 28:98-101. 10.1016/j.pdpdt.2019.08.028

Alves, F., Carmello, J.C., Alonso, G.C., Mima, E.G.D.O., Bagnato, V.S., and Pavarina, A.C. 2020. A randomized clinical trial evaluating Photodithazine-mediated Antimicrobial Photodynamic Therapy as a treatment for Denture stomatitis. Photodiagnosis and Photodynamic Therapy 32:102041. 10.1016/j.pdpdt.2020.102041

Conti, H.R., Shen, F., Nayyar, N., Stocum, E., Sun, J.N., Lindemann, M.J., Ho, A.W., Hai, J.H., Yu, J.J., Jung, J.W., Filler, S.G., Masso-Welch, P., Edgerton, M., and Gaffen, S.L. 2009. Th17 cells and IL-17 receptor signaling are essential for mucosal host defense against oral candidiasis. J Exp Med 206:299-311. 10.1084/jem.20081463

Conti, H.R., and Gaffen, S.L. 2010. Host responses to Candida albicans: Th17 cells and mucosal candidiasis. MICROBES AND INFECTION 12:518-527. 10.1016/j.micinf.2010.03.013

de Senna, A.M., Vieira, M.M.F., Machado-de-Sena, R.M., Bertolin, A.O., Nez, S.C.N., and Ribeiro, M.S. 2018. Photodynamic inactivation of Candida ssp. on denture stomatitis. A clinical trial involving palatal mucosa and prosthesis disinfection. Photodiagnosis and Photodynamic Therapy 22:212-216.

Deeds, M.C., Anderson, J.M., Armstrong, A.S., Gastineau, D.A., Hiddinga, H.J., Jahangir, A., Eberhardt, N.L., and Kudva, Y.C. 2011. Single dose streptozotocin-induced diabetes:

considerations for study design in islet transplantation models. LABORATORY ANIMALS 45:131-140. 10.1258/la.2010.010090
Gendreau, L., and Loewy, Z.G. 2011. Epidemiology and etiology of denture stomatitis. Journal of Prosthodontics-Implant Esthetic and Reconstructive Dentistry 20:251-260. 10.1111/j.1532-849X.2011.00698.x
Gu, Y., Zhang, L., Liu, J., Zhang, X., Liu, N., and Liu, Q. 2022. An Experimental Study of Photoactivated Disinfection in the Treatment of Acute Pseudomembranous Stomatitis. PHOTOCHEMISTRY AND PHOTOBIOLOGY 98:1418-1425. 10.1111/php.13637
Javed, F., Klingspor, L., Sundin, U., Altamash, M., Klinge, B., and Engstrom, P.E. 2009. Periodontal conditions, oral Candida albicans and salivary proteins in type 2 diabetic subjects with emphasis on gender. BMC Oral Health 9:12. 10.1186/1472-6831-9-12
Johnson, C.C., Yu, A., Lee, H., Fidel, P.L., and Noverr, M.C. 2012. Development of a Contemporary Animal Model of Candida albicans-Associated Denture Stomatitis Using a Novel Intraoral Denture System. INFECTION AND IMMUNITY 80:1736-1743. 10.1128/IAI.00019-12
King, A.J. 2012. The use of animal models in diabetes research. Br J Aesthet:877-894. 10.1111/j.1476-5381.2012.01911.x
Konopka, K., and Goslinski, T. 2007. Photodynamic Therapy in Dentistry. JOURNAL OF DENTAL RESEARCH 86:694-707. 10.1177/154405910708600803
Lee, J.S., Tato, C.M., Joyce-Shaikh, B., Gulen, M.F., Cayatte, C., Chen, Y., Blumenschein, W.M., Judo, M., Ayanoglu, G., McClanahan, T.K., Li, X., and Cua, D.J. 2015. Interleukin-23-Independent IL-17 Production Regulates Intestinal Epithelial Permeability. IMMUNITY 43:727-738. 10.1016/j.immuni.2015.09.003
Lie Tobouti, P., Casaroto, A.R., de Almeida, R.S.C., de Paula Ramos, S., Dionísio, T.J., Porto, V.C., Santos, C.F., and Lara, V.S. 2016. Expression of Secreted Aspartyl Proteinases in an Experimental Model of Candida albicans-Associated Denture Stomatitis. Journal of prosthodontics 25:127-134. 10.1111/jopr.12285
Matsuzaki G, U.M. 2018. Interleukin-17 family cytokines in protective immunity against infections: role of hematopoietic cell-derived and non-hematopoietic cell-derived interleukin-17s. Running title: IL-17 family cytokines in infections. MICROBIOLOGY AND IMMUNOLOGY 62:1-13. 10.1111/1348
McGeachy, M.J., Cua, D.J., and Gaffen, S.L. 2019. The IL-17 Family of Cytokines in Health and Disease. IMMUNITY 50:892-906. 10.1016/j.immuni.2019.03.021
Mima, E.G., Pavarina, A.C., Silva, M.M., Ribeiro, D.G., Vergani, C.E., Kurachi, C., and Bagnato, V.S. 2011. Denture stomatitis treated with photodynamic therapy: five cases. Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, and Endodontology 112:602-608. 10.1016/j.tripleo.2011.05.019
Mima, E.G., Vergani, C.E., Machado, A.L., Massucato, E.M., Colombo, A.L., Bagnato, V.S., and Pavarina, A.C. 2012. Comparison of Photodynamic Therapy versus conventional

antifungal therapy for the treatment of denture stomatitis: a randomized clinical trial. Clin Microbiol Infect 18:E380-E388. 10.1111/j.1469-0691.2012.03933.x

Pinto, A.P., Rosseti, I.B., Carvalho, M.L., Da Silva, B.G.M., Alberto-Silva, C., and Costa, M.S. 2018. Photodynamic Antimicrobial Chemotherapy (PACT), using Toluidine blue O inhibits the viability of biofilm produced by Candida albicans at different stages of development. Photodiagnosis and Photodynamic Therapy 21:182-189. 10.1016/j.pdpdt.2017.12.001

Robert, M., and Miossec, P. 2017. Effects of Interleukin 17 on the cardiovascular system. AUTOIMMUNITY REVIEWS 16:984-991. 10.1016/j.autrev.2017.07.009

Saijo, S., Ikeda, S., Yamabe, K., Kakuta, S., Ishigame, H., Akitsu, A., Fujikado, N., Kusaka, T., Kubo, S., Chung, S.H., Komatsu, R., Miura, N., Adachi, Y., Ohno, N., Shibuya, K., Yamamoto, N., Kawakami, K., Yamasaki, S., Saito, T., Akira, S., and Iwakura, Y. 2010. Dectin-2 recognition of alpha-mannans and induction of Th17 cell differentiation is essential for host defense against Candida albicans. IMMUNITY 32:681-691. 10.1016/j.immuni.2010.05.001

Schönherr, F.A., Sparber, F., Kirchner, F.R., Guiducci, E., Trautwein-Weidner, K., Gladiator, A., Sertour, N., Hetzel, U., Le, G.T.T., Pavelka, N., D'Enfert, C., Bougnoux, M., Corti, C.F., and LeibundGut-Landmann, S. 2017. The intraspecies diversity of C. albicans triggers qualitatively and temporally distinct host responses that determine the balance between commensalism and pathogenicity. Mucosal Immunology 10:1335-1350. 10.1038/mi.2017.2

Scwingel, A.R., Barcessat, A.R.P., Núñez, S.C., and Ribeiro, M.S. 2012. Antimicrobial Photodynamic Therapy in the Treatment of Oral Candidiasis in HIV-Infected Patients. PHOTOMEDICINE AND LASER SURGERY 30:429-432. 10.1089/pho.2012.3225

SM, M., D, M., L, M., A, P., PS, A., M, M., and A., B. 2014. Glucose directly promotes antifungal resistance in the fungal pathogen, Candida spp. Biol. Chem 289:25468-25473.

Soysa, N.S., Samaranayake, L.P., and Ellepola, A.N.B. 2006. Diabetes mellitus as a contributory factor in oral candidosis. DIABETIC MEDICINE 23:455-459. 10.1111/j.1464-5491.2005.01701.x

Sugio, C.Y.C., Garcia, A.A.M.N., Albach, T., Moraes, G.S., Bonfante, E.A., Urban, V.M., and Neppelenbroek, K.H. 2020. Candida-Associated Denture Stomatitis and Murine Models: What Is the Importance and Scientific Evidence? Journal of Fungi 6:70. 10.3390/jof6020070

Yano, J., Yu, A., Fidel, P.L., and Noverr, M.C. 2016. Transcription Factors Efg1 and Bcr1 Regulate Biofilm Formation and Virulence during Candida albicans-Associated Denture Stomatitis. PLoS One 11:e159692. 10.1371/journal.pone.0159692

Zhang, L., Hu, Q., Zhang, Y., Wang, Y., Liu, N., and Liu, Q. 2023. Rapid Inactivation of mixed biofilms of Candida albicans and Candida tropicalis using antibacterial photodynamic therapy: Based on PAD™ Plus. Heliyon 9:e15396. 10.1016/j.heliyon.2023.e15396

Zimmet, P., Alberti, K.G., Magliano, D.J., and Bennett, P.H. 2016. Diabetes mellitus statistics on prevalence and mortality:facts and fallacies. Nature Reviews Endocrinology 12:616-622.

Table 1(on next page)

Average optical density values of IL-17 of each group after treatment

Table 1. Average optical density values of IL-17 of each group after treatment

Group	N	IL-17 average optical density values $\bar{x}\pm s$		*t* value	*P* value
		1d	7d		
infection group	3	0.1051±0.0052	0.1201±0.0105	-1.722	0.227
PAD-1 group	3	0.0575±0.0040[a]	0.1158±0.0251	-4.525	0.046
PAD-2 group	3	0.0414±0.0020[ab]	0.0630±0.0056[ab]	-7.291	0.018
NYS group	3	0.0416±0.0057[ab]	0.0680±0.0055[ab]	-4.719	0.042

(a) compared with infection group, $P<0.05$; (b)compared with PAD-1 group, $P<0.05$

Table 2(on next page)

Average optical density values of TNF-α of each group after treatment

Table 2. Average optical density values of TNF-α of each group after treatment

Group	N	TNF-α average optical density values $\bar{x}\pm s$		*t* value	*P* value
		1d	7d		
infection group	3	0.0723±0.0004	0.0756±0.0006	-6.043	0.026
PAD-1 group	3	0.0653±0.0018[a]	0.0732±0.0031	-10.678	0.009
PAD-2 group	3	0.0640±0.0002[a]	0.0659±0.0011[ab]	-2.876	0.103
NYS group	3	0.0665±0.0037[a]	0.0672±0.0025[ab]	-1.958	0.189

(a)compared with infection group, $P<0.05$; (b)compared with PAD-1 group, $P<0.05$

Figure 1

Figure 1 Denture fabrication

(A)Impression of the palate. (B)A corresponding gypsum model. (C)The light-cured acrylic denture.

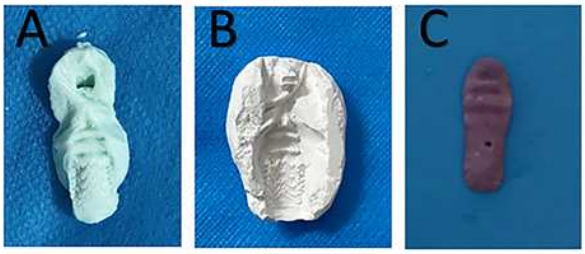

Figure 2

Figure 2 Palate inflammation lesions of rats after treatment

(A-D) Infection group, PAD-1 group, PAD-2 group, NYS group of 1d after treatment. (E-H) Infection group, PAD-1 group, PAD-2 group, NYS group of 7d after treatment.

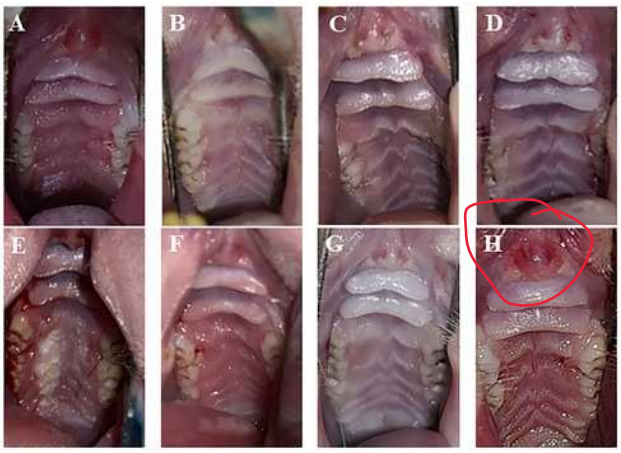

Figure 3

Figure 3 Palate inflammation score of rats after treatment

(A)1d after treatment. (B)7d after treatment. (N=6)

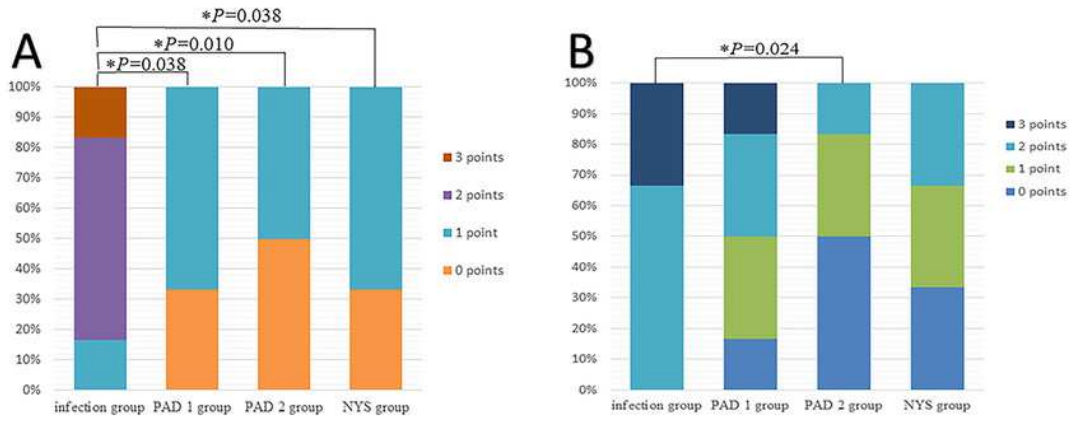

Figure 4

Figure 4 Comparison of palate and denture fungal ~~load~~ at 1 d and 7 d after treatment

(A)palate fungal ~~load.~~ (B)denture fungal ~~load.~~ (N=6)

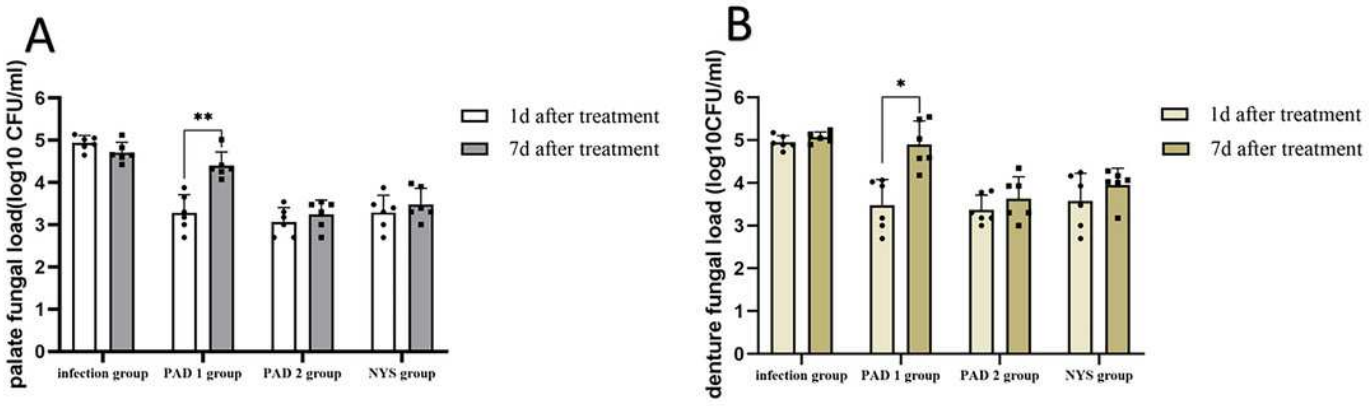

Figure 5

Figure 5 Palate fungal load of each group after treatment

(A)1d after treatment. (B)7d after treatment. (N=6)

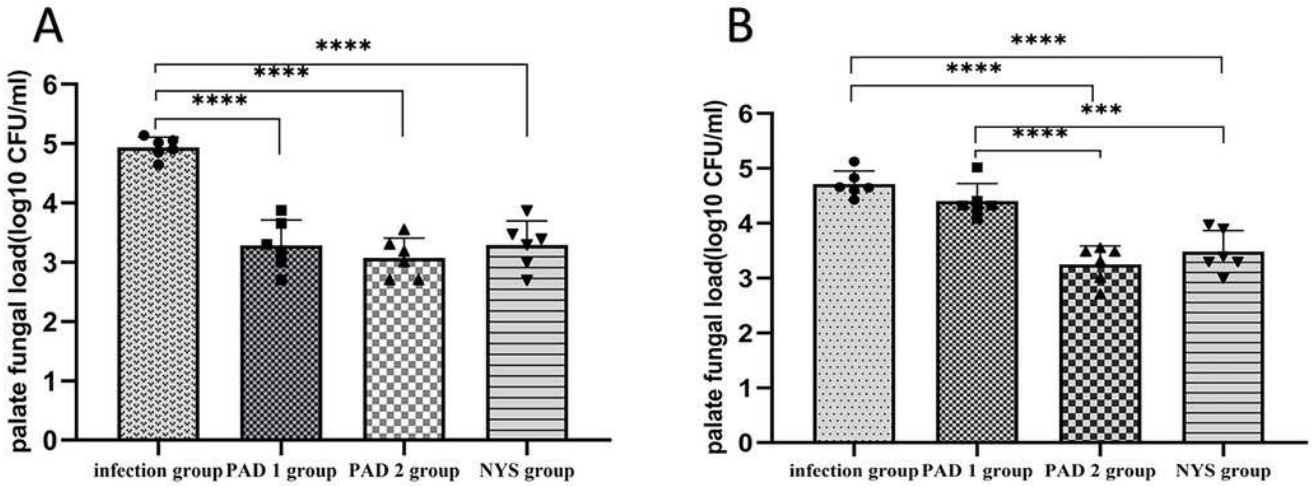

Figure 6

Figure 6 Denture fungal ~~load~~ of each group after treatment

(A)1d after treatment. (B)7d after treatment. (N=6)

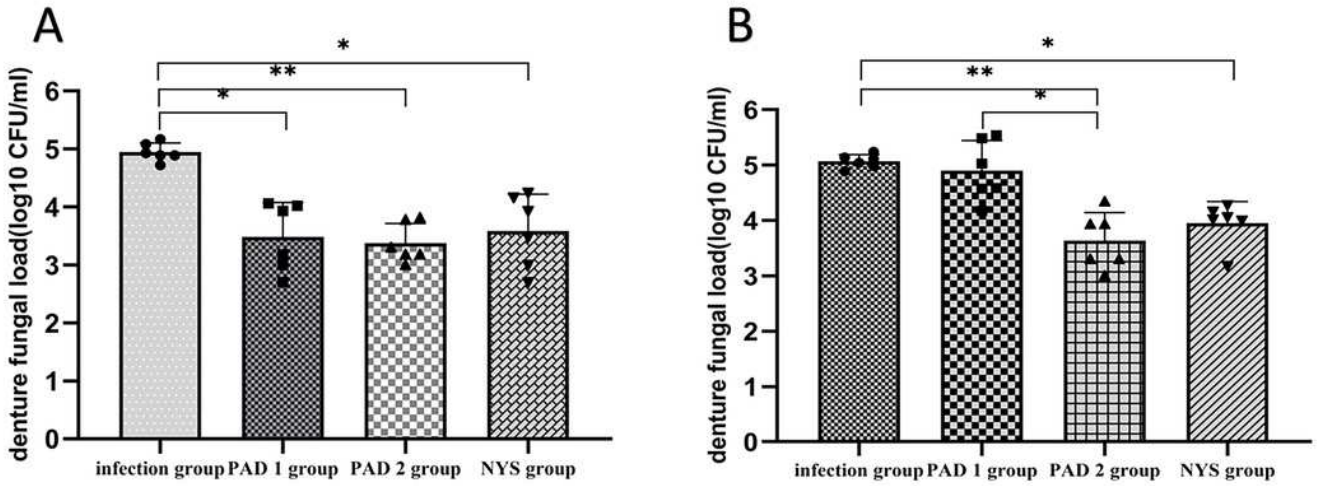

Figure 7

Figure 7 HE staining of palate mucosa of each group after treatment

(A–D) Infection group, PAD-1 group, PAD-2 group, NYS group of 1d after treatment. (E–H) Infection group, PAD-1 group, PAD-2 group, NYS group of 7d after treatment.

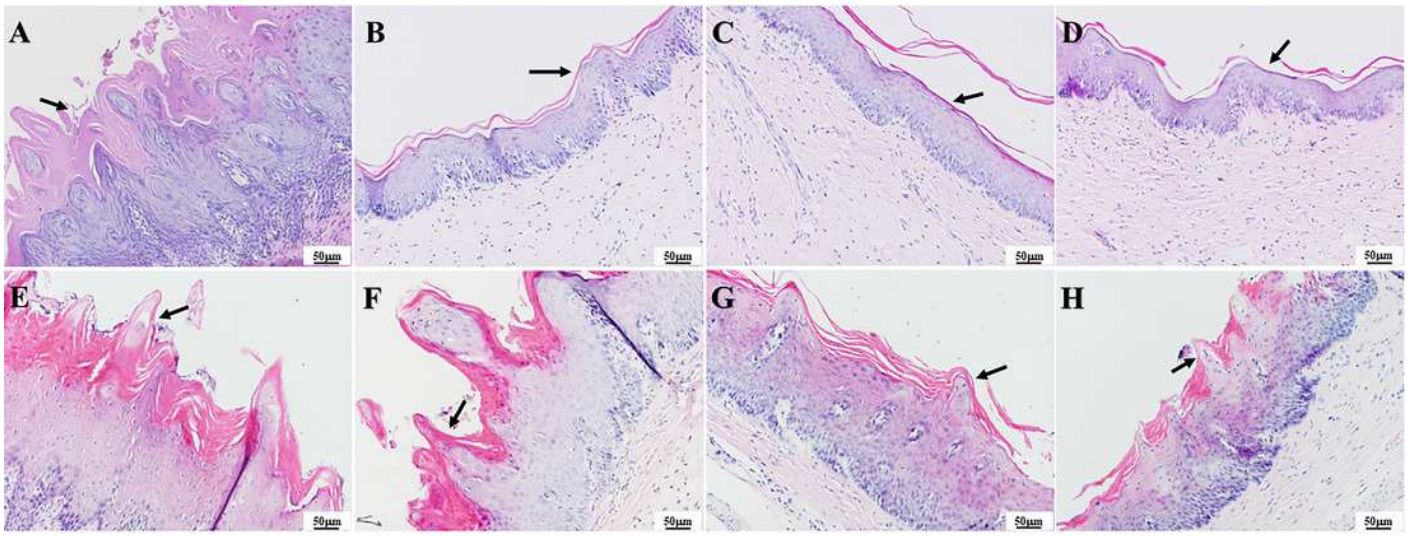

Figure 8

Figure 8 PAS staining of palate mucosa of each group after treatment

(A–D) Infection group, PAD-1 group, PAD-2 group, NYS group of 1d after treatment. (E–H) Infection group, PAD-1 group, PAD-2 group, NYS group of 7d after treatment.

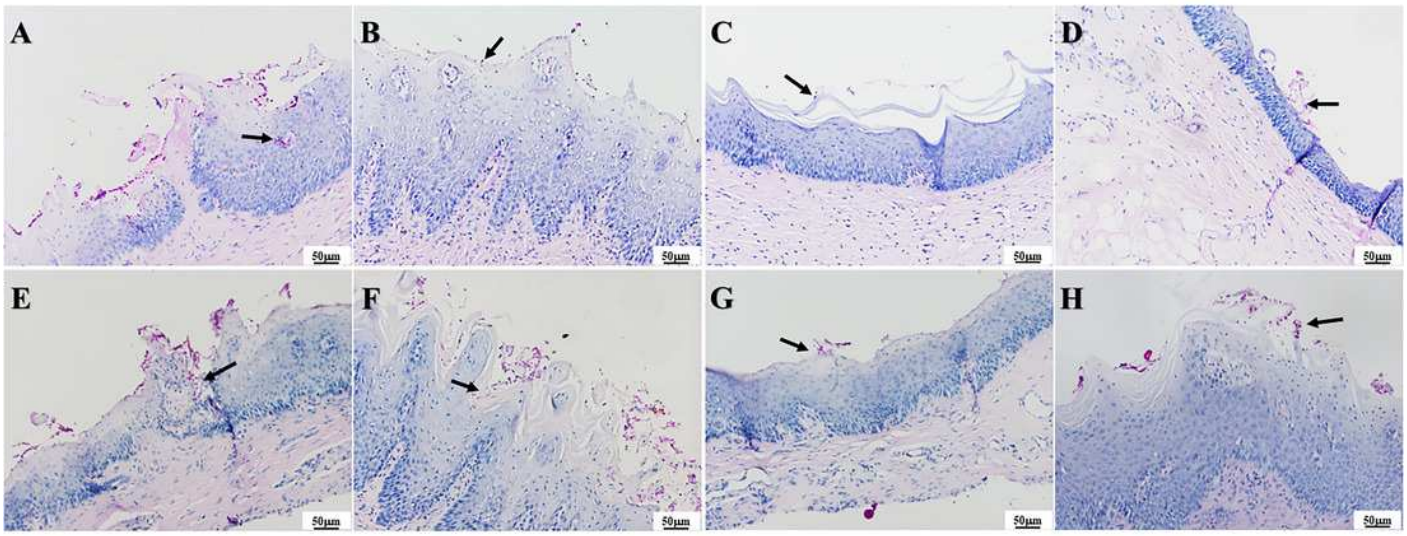

Figure 9

Figure 9 Expression of IL-17 in the palate mucosa of each group after treatment

(A–D) Infection group, PAD-1 group, PAD-2 group, NYS group of 1d after treatment. (E-H) Infection group, PAD-1 group, PAD-2 group, NYS group of 7d after treatment.

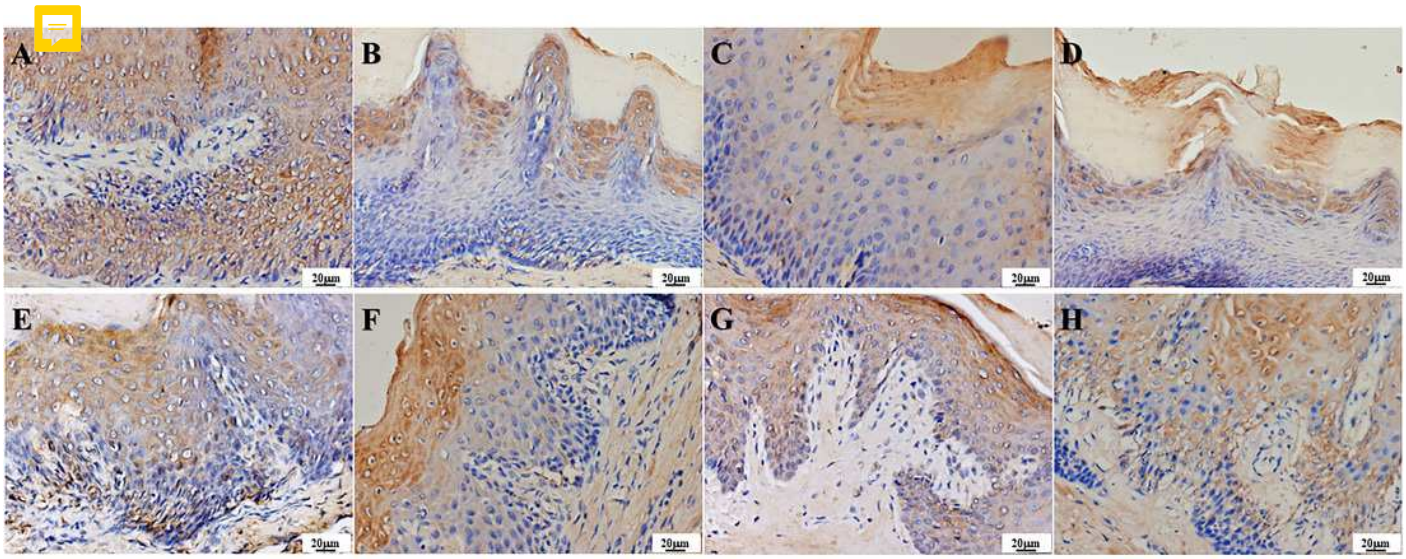

Figure 10

Figure 10 Expression of TNF-α in the palate mucosa of each group after treatment

(A–D) Infection group, PAD-1 group, PAD-2 group, NYS group of 1d after treatment. (E–H) Infection group, PAD-1 group, PAD-2 group, NYS group of 7d after treatment.