Analysis of the Therapeutic Effect of Nano-Silver on Periodontitis and Its Influence on Disease Recurrence

Hui Wang
Lulu Wang
Tian Chao
Shumei Qi

*Hui Wang,Department of Stomatology for Children, Jining Stomatological Hospital, Jining 272001, Shandong, PR China, Lulu Wang and Chao Tian Department of Stomatology, The No. 8 People's Hospital of Qingdao, Qingdao 266100, Shandong, PR China, Shumei Qi*Department of Stomatology, Jinan Maternal and Child Health Hospital, Jinan 250001, Shandong, PR China*Corresponding author: Department of Stomatology, Jinan Maternal and Child Health Hospital, Jinan 250001, Shandong, PR China*

Periodontitis is a chronic oral inflammatory disease that is difficult to treat and is therefore the subject of clinical research seeking new and effective treatment. Nano-silver has antibacterial and anti-inflammatory effects; however, its application in periodontitis is not well studied. In this study, we show decreases in periodontitis-associated inflammatory cytokines interleukin IL-4, IL-6, IL-8, IL-10, and tumor necrosis factor alpha (TNF-a) ($P < 0.05$) and in oxidative stress ($P < 0.05$) under intervention with nano-silver solution. The protein expression of CCL21, heat-shock protein 90 (HSP90), andE-selectin in periodontal tissues decreased ($P < 0.05$), while bone structure improved ($P < 0.05$). This work suggests that nano-silver solution can effectively inhibit the inflammatory response and oxidative stress response of periodontitis and improve the periodontal tissue and tooth structure to some extent; it may provide a new periodontitis treatment in the future.

Keywords: Nano-Silver Solution, Periodontitis, Inflammatory Cytokines, Oxidative Stress.
Tob Regul Sci.™ 2021;7(5):1131-1137
DOI: doi.org/10.18001/TRS.7.5.31

INTRODUCTION

Periodontitis is a chronic oral disease with a very high inci- dence worldwide [1]. According to current research, peri- odontitis has been detected in 69.9% of people aged 55–64 in China, and its incidence is increasing with declining oral hygiene habits [2, 3]. Moreover, there are no particular symptoms of early-stage periodontitis, so patients easily ignore this [4, 5]. At present, the clinical treatment of peri- odontitis mainly includes gingival cleaning and scaling, periodontal pocket cleaning, and stabilizing loose teeth [6]. In clinical practice, different or even multiple targeted treatments are required according to the patient's condi- tions. Not only is the treatment cycle relatively long, but most patients experience recurrence [7, 8]. In view of the limitations of the current treatment of periodontitis and the extent of its incidence in the population, it is urgent that a new and effective clinical treatment befound.

Nano-silver has an extremely stable molecular struc- ture, a long half-life, and better human metabolic rate, all of which confer advantages over the use of multiple drugs [9–11]. It is an extremely significant antibacterial and anti-inflammatory substance that has proven effective in wound repair and as an antibacterial treatment in clinical practice [12–22]. Rao et al. showed that nano-silver has a significant effect on arthritis rats [23]. Periodontitis,whichis caused by the inflammatory changes in the periodontal tissues [24], might also be affected by nano-silver; how- ever, research confirming this has been conducted. Here we aimed to confirm the therapeutic effect of nano-silver on periodontitis through experiments and provide a reliable theoretical basis for future clinical treatment of patients withperiodontitis.

MATERIALS ANDMETHODS

Animals

A total of 40 Wistar rats weighing 280–320 g were pur- chased from Beijing Shanyi zhengzi

pharmaceutical tech- nology Co., Ltd.; the animal use license number was SYXK (Beijing) 2020-0016. Five rats were kept in one cage at 18 °C–25°C.

Periodontitis Modeling

The rats were divided into four groups (n = 10), with two groups used to model periodontitis. Chloral hydrate(10%)

was intraperitoneally injected into anesthetized rats for the modeling groups, and a stainless steel wire was inserted through the junction of the first and second molars and attached to the neck of the teeth. Rats were fed with high sugar and sticky milk for 8 days and then fed normally. Four weeks later, the gingival swelling, bleeding, attach- ment of food residues to the epithelium, andformationof subosseous pockets of the first molars were observed in all rats; this was considered a successful model of periodontitis.

Preparation of Nano-Silver Solution

Nano-silver was purchased from Shanghai Superwei Nan- otechnology; 0.1 g was added to 100 mL deionized water with ultrasonic oscillation mixing to create 0.1% (1 mg/mL) nano-silver solution. The release curve of nano-silver was measured by ultraviolet spectrophotometer, and the morphological characteristics of the nano-silver solu- tion were microscopicallyobserved.

RatTreatment

Experimental rats were divided randomly into two groups. For the model A group (n =10), periodontal irrigation was performed with 2 mL nano-silver solution twice a day for 1 week. The model B group was treated in the same way but with 2 mL normal saline. “Normal” rats were treated in the same way as the modeling, with nano-silver solution used for washing in group A and normal saline in groupB.

Detection of Inflammatory Cytokines

Serum inflammatory cytokines interleukin IL-4, IL-6, IL- 8, and IL-10 and tumor necrosis factor alpha (TNF-a) were detected using ELISA in nano-silver solution before flushing, nano-silver solution after flushing at 4 weeks, and extracted neck vein blood of rats; in each case, 2mL

sample was collected in a coagulation tube, left at room temperature for 30 min, and then centrifuged (400× g, 4 °C) for 10 min before serumanalysis.

Detection of Oxidative Stress

As above, venous blood was extracted from rats before and after irrigation; malondialdehyde (MDA) was detected by the thiobarbituric acid method and in serum. The kit was purchased from Beijing Solebao Biological Technol- ogy Co., Ltd. Immunofluorescence assay for reactive oxy-gen species (ROS) was used. The kit was purchased from Beijing Baiolaibo Technology Co., Ltd. For thexanthineoxidase method, the superoxide dismutase (SOD) kit was purchased from Beijing Box Manufacturing Technology Co., Ltd.

Protein Detection

After 4 weeks, all rats were killed under anesthesia, and the periodontal tissue was removed. Chemokine CCL21, heat-shock protein 90 (HSP90), and E-selectin protein expressions in the periodontal tissues were detected by westernblot.

Dental Histomorphometry

Micro-CT was used to examine the bone images at the roots of molars in molar tissue samples, and bone tissue parameters, such as bone volume (BV/TV), bone trabecu- lar thickness (Tb.Th), and bone trabecular number (Tb.N), wereanalyzed.

StatisticalMethod

SPSS 22.0 statistical software was used for statistical anal- yses. One-way ANOVA was used for a LSD postmortem test to determine the differences among all groups, along with paired t-test both pretherapy and posttreatment. The results were statistically significant ($P < 0.05$).

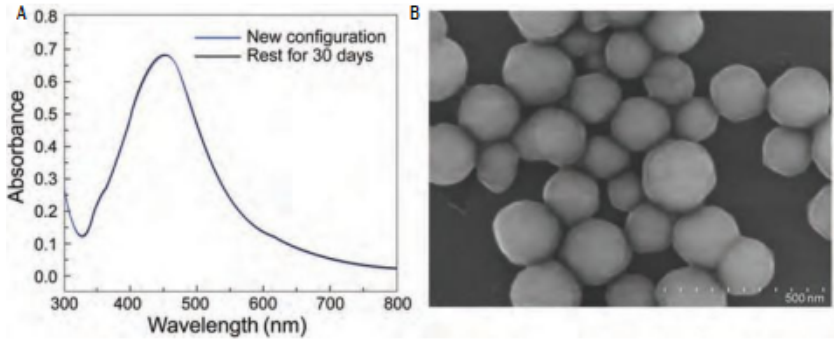
RESULTS AND DISCUSSION

Preparation Effect of Nano-Silver Solution Absorbance of nano-silver solution by UV spectrophotom- etry steadily decreased, confirming that nano-silver solu- tion was released and had a long half-life (Fig. 1(A)). Microscopic analysis of nano-silver solution showed a spherical shape with a size of about 150–250 nm, asmooth surface and uniform distribution, and a relatively neat dis- tribution structure (Fig.1(B)).

Contrast Inflammatory Cytokines

No differences were detected in IL-4, IL-6, IL-8, IL- 10, and TNF-a between models A and B in pretreat- ment, in either control or experimental groups ($P > 0.05$).

Fig. 1.
Nano-silver solution morphology. (A) Release curve; (B) nano-silver morphology.



After treatment, IL-4, IL-6, IL-8, IL-10, and TNF-a of model A rats were lower than of model B rats but higher than those of control A and B groups (Figs. 2(A–E)) (**P** <0.05). The inflammatory cytokines in model A were all lower than before treatment, while those in model B were higher (Figs. 2(A–E)) (**P** <0.05). IL-4, IL-6,IL-

8, IL-10, and TNF-a showed no differences after treat- ment between control A and B groups (Figs. 2(A–E)) (**P**>0.05).

Comparison of OxidativeStress

There was no difference detected in MDA, ROS, and SOD in models A and B, and no difference in controls A and B before treatment (Figs. 3(A–C)) (**P** >0.05). After cure, MDA and ROS were lower in model A than in model B, but SOD was increased in model A (Figs. 3(A–C)) (**P** <0.05). MDA and ROS in model A were lower, while SOD was higher than before treatment (**P** <0.05). MDA and ROS were lower in model B (Figs. 3(A–C)) (**P** <0.05).

Fig.2.
Comparisonofinflammatorycytokines.(A)ComparisonofIL-4concentration;(B)IL-6concentrationcontrast;(C)IL-8concentrationcontrast;
(D) IL-10 concentration contrast; (E) TNF-a concentration contrast. Contrast the same group before cure, *P <0.05; contrast model A aftertreatment,
#P <0.05; contrast model B after treatment, &P <0.05.

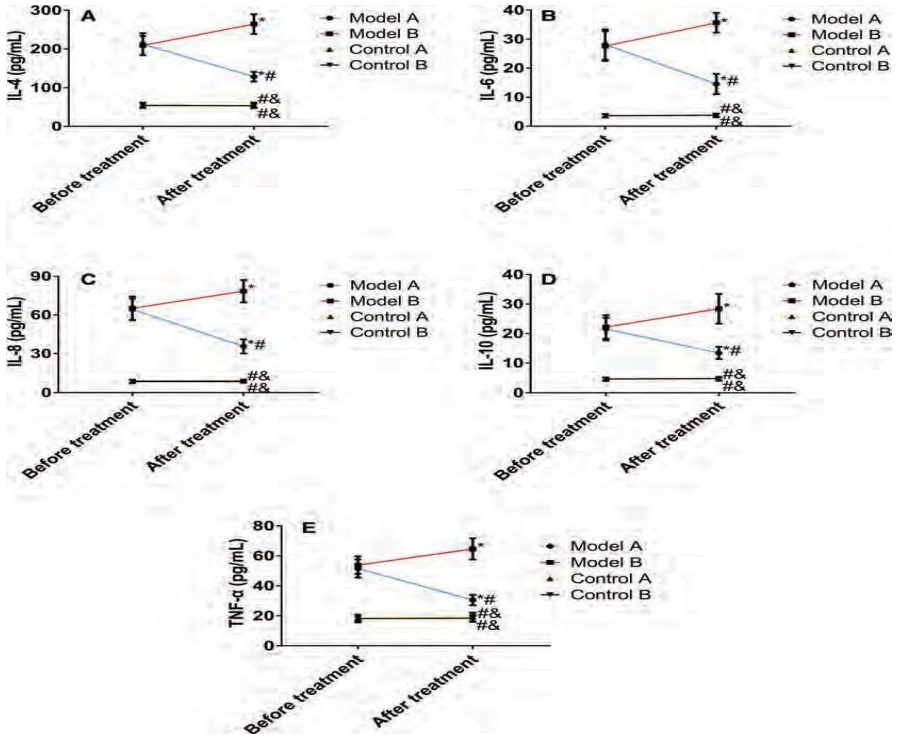
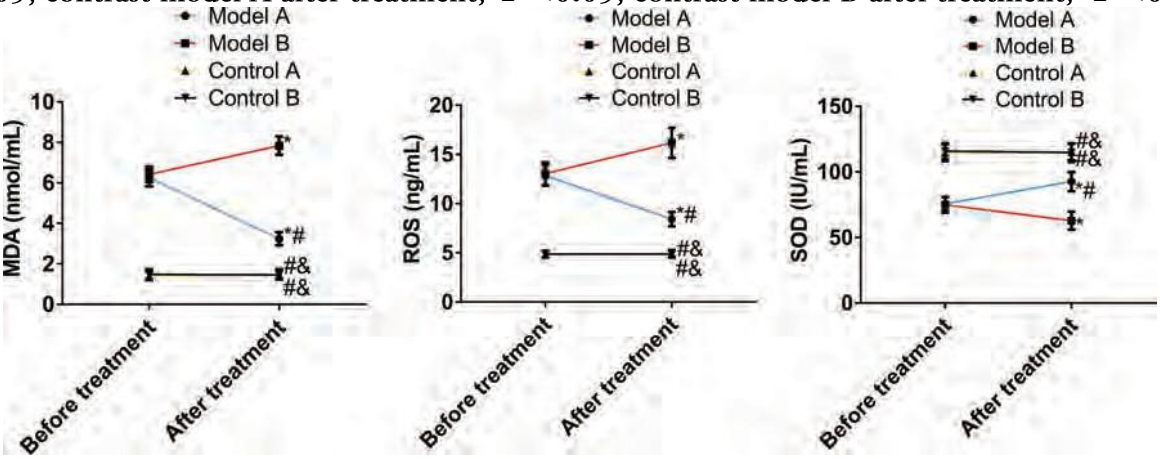


Fig. 3.
Comparison of oxidative stress. (A) Comparison of MDA concentration; (B) comparison of ROS concentration; (C) comparison of SOD concentration. Contrast the same group before cure, *P <0.05; contrast model A after treatment, #P <0.05; contrast model B after treatment, &P <0.05.



Protein Expression Comparison

Protein expression of CCL21, HSP90, and E-selectin in model A was greater than in model B (Figs. 4(A–D)) (P <0.05). No differences were found between control groups in CCL21, HSP90, and E-selectin protein expression (Figs. 4(A–D)) (P >0.05).

Comparison of ToothHistomorphology

BV/TV, Tb.Th, and Tb.N were greater in model A than in model B (Figs. 5(A–C)) (P <0.05), with BV/TV of model A being the same as in control A and B (Fig. 5(A)), but Tb.Th and Tb.N were lower than in controls A and B (Figs. 5(B and C)) (P <0.05).

Fig. 4.
Protein expression comparison. (A) Protein imprinting map; (B) CCL21 protein expression; (C) HSP90 protein expression; (D) E-selectin protein expression. Contrast model A, *P <0.05; contrastmodel B, #P <0.05.

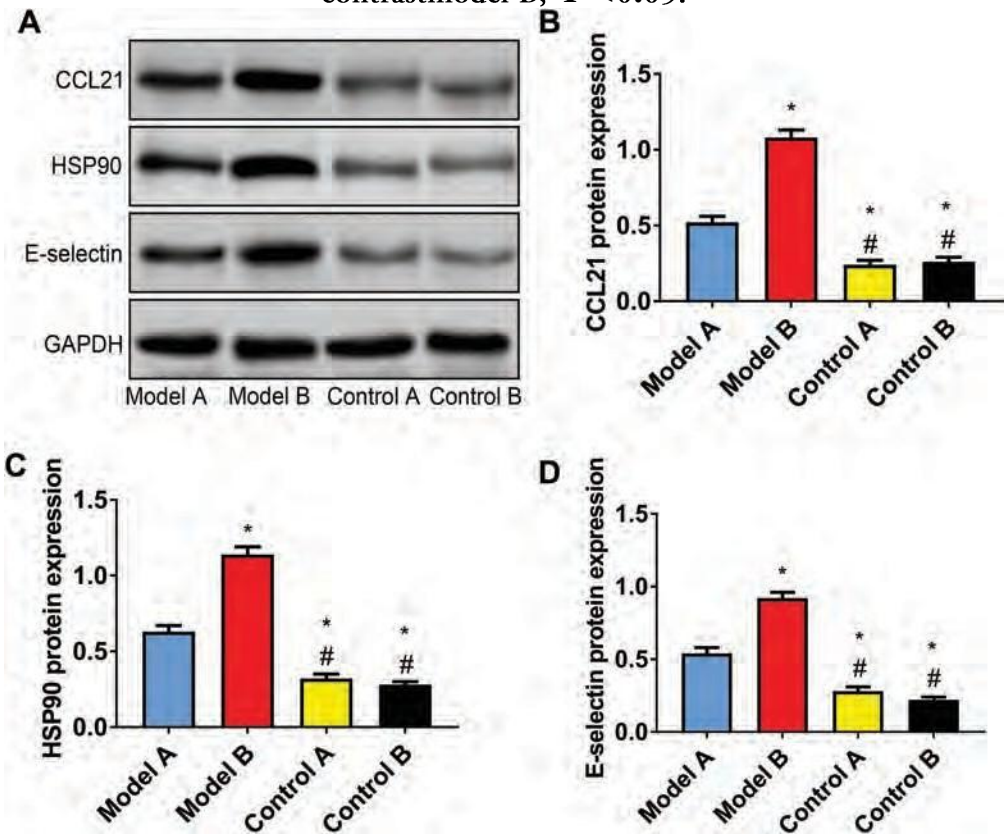
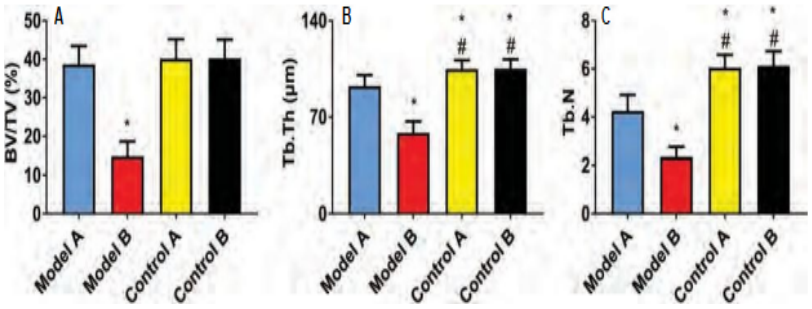


Fig. 5.
Comparison of bone histomorphology. (A) BV/TV comparison; (B) Tb.Th comparison; (C) Tb.N comparison. Contrast model A, *P <0.05; contrast model B, #P <0.05.



Discussion

A new treatment could greatly improve the clinical cure rate and prognosis for periodontitis [25]. The anti- inflammatory and antimicrobial effects of nano-silver solution have been confirmed in clinical practice [26], along with its relatively consistent and targeted effect on periodontitis.

To determine the effect of nano-silver on periodontitis, we first measured the inflammatory cytokines in rats. The results showed that the inflammatory factors of periodon- titis in rats were significantly inhibited after rinsing with nano-silver solution. The results were also consistent with the previous studies on the application of nano-silver, con- firming that nano-silver has significant anti-inflammatory effects in periodontitis. IL-6, IL-8, IL-10, and TNF-a, the common inflammatory cytokines in clinical studies, play an important role in periodontitis [27, 28]. Studies have confirmed that periodontal cells and tissues can release a large number of inflammatory mediators during the occur- rence of periodontitis, causing tissue damage, oxidation, and necrosis [29]. Therefore, inhibiting the release of inflammatory mediators in the treatment of periodontitis is the key. Nano-silver has a remarkable effect in this respect. In addition, we measured the changes of oxidative stress response in rats, and the results also showed that, under the intervention of nano-silver solution, MDA and ROS were reduced in rats with periodontitis, while SOD was increased. Previous research has shown that MDA, SOD, and ROS are sensitive indicators of oxidative stress response [30]. Elevation of MDA and decrease of SOD indicate that cells under certain stimulation produce a large number of free radicals and ROS, which cause cell damage and necrosis, thus causing diseases [31]. Given our anal- yses and previous studies, we know that the release of a large amount of inflammatory mediators destroys the bal- ance of oxidative stress in periodontal tissues, damages the gingival tissues, increases the susceptibility of periodontal tissues, damages the local immunity of periodontal tissues, and causes periodontal tissue damage during the onset of periodontitis [32]. In this environment, it is also conducive to the invasion of anaerobes and toxins and increasestheincidence of oral infectious diseases [33]. If thisprocessisnot controlled and treated in time, it may causemoreseri-ous periodontal injury. Applying nano-silversolutioncaneffectively reduce oxidative stress injury inratperiodontaltissues, creating possibility for futureclinicalapplication.To further understand the effect of nano-silversolutiononperiodontitis, we also detected CCL21, HSP70,andE-selectin protein in periodontal tissues of rats ineachgroup.Previous studies have confirmed that CCL21,asachemokine, has the dual role of mediatingcelladhesionand cell migration. When abnormal changes occurintis-sues, CCL21 can cause inflammatory cytokinestoaccu-mulate in large quantities and causeinflammatorycascadereaction [34]. HSP90 is one of the mostsensitivemem-bers of the HSP family. As a highlyconservedprotein,HSP90 is synthesized in large quantities understressstim-ulation and improves the stress tolerance of cells[35].E-selectins have a similar effect to CCL21, whichcanpro-mote the adhesion of endothelial cells andwhitebloodcells, causing a vascular reaction [36]. Allthreeproteinshave been proved to have significant functionsinperi-odontitis [37–39]. The elevated expressionindicatestheintensification of periodontal inflammatoryresponse.Inthis study, after the intervention of nano-silversolutiononperiodontitis rats, we found that CCL21, HSP70,andE-selectins in rats were reduced, suggestingthatnano-silversolution could inhibit the expression ofCCL21,HSP70,and E-selectins, and once again, inflammationinhibitedtheinflammatory response inhibition effect onperiodontaltis-sues of periodontitis. Finally, we compared thedentaltis-sue of the rats in each group with micro-CT,whichshowedthat the dental tissue structure of the ratswithperiodon-titis was significantly improved after theinterventionofnano-silver solution. Micro-CT, as aclinicallyrecognizedinstrument for the

evaluation of periodontalboneregener-ation, can accurately display relevant informationintoothstructure [40]. Our experimental results showthatnano-silver not only has a good inhibitory effectonperiodontalinflammation and oxidative stress but also canimprovetheintegrity of the tooth structure. In the future, it mayhavea certain improvement effect not only on periodontitis but also on other periodontal diseases.

We also used nano-silver solution in normal rats and found that there was no adverse reaction in normal rats, indicating that nano-silver solution is safe and worthy of clinical application.

However, since we did not use human subjects, there may be some errors between the specific application of nano-silver and the results of our animal experiments. In addition, as no studies on the effect of nano-silver on peri- odontitis have been conducted, the concentrations chosen here were based on previous studies, and more in-depth experimental analysis is needed to confirm the optimal drug use. However, the exact mechanism of nano-silver solution on periodontitis remains incompletely clear; there- fore, more research is needed. We hope that this study can provide a better reference basis for the future application of nano-silver in periodontitis and attract more researchers to carry out subsequent experimentalanalysis.

CONCLUSION

Nano-silver solution can effectively inhibit the inflamma- tory response and oxidative stress response of periodontitis and improve the periodontal tissue and tooth structure to some extent. It may be a new direction of periodontitis treatment in the future.

REFERENCES AND NOTES

1. Slots, J., 2017. Periodontitis: Facts, fallacies and the future. *Peri- odontology 2000, 75*(1),pp.7–23.
2. Bartold, P.M., 2018. Lifestyle and periodontitis: The emergenceofpersonalized periodontics. *Periodontology 2000, 78*(1), pp.7–11.
3. Kumar, S., 2019. Evidence-based update on diagnosis and manage- ment of gingivitis and periodontitis. *Dental Clinics of North Amer- ica, 63*(1),pp.69–81.
4. Graziani, F., Karapetsa, D., Alonso, B. and Herrera, D., 2017. Non- surgical and surgical treatment of periodontitis: How many options for one disease? *Periodontology 2000, 75*(1),pp.152–188.
5. Li, C., Lv, Z., Shi, Z., Zhu, Y., Wu, Y., Li, L. and Iheozor-Ejiofor, Z., 2017. Periodontal therapy for the management of cardiovascular dis- ease in patients with chronic periodontitis. *The Cochrane Database of Systematic Reviews*, (11),pp.1-46.
6. Cobb, C.M., 2017. Lasers and the treatment of periodontitis: The essence and the noise. *Periodontology 2000, 75*(1),pp.205–295.
7. daCosta,L.F.N.P.,Amaral,C.D.S.F.,Barbirato,D.D.S.,Leã o,A.T.T.and Fogacci, M.F., 2017. Chlorhexidine mouthwash as an adjunct to mechanical therapy in chronic periodontitis: A meta-analysis. *Jour- nal of the American Dental Association, 148*(5), pp.308–318.
8. Sanz, M., Marco Del Castillo, A., Jepsen, S., Gonzalez-Juanatey, J.R., D'Aiuto, F., Bouchard, P., Chapple, I., Dietrich, T., Gotsman, I., Graziani, F., Herrera, D., Loos, B., Madianos, P., Michel, J.B., Perel, P.,Pieske,B.,Shapira,L.,Shechter,M.,Tonetti,M.,Vlachopo ulos,C. and Wimmer, G., 2020. Periodontitis and cardio- vascular diseases: Consensus report. *Journal of Clinical Periodon- tology, 47*(3), pp.268–288.
9. Ye, J.H. and Augustin, M.A., 2019. Nano- and micro-particlesfordelivery of catechins: Physical and biological performance. *Critical Reviews in Food Science and Nutrition, 59*(10), pp.1563–1579.
10. Choi, D., Montermini, L., Jeong, H., Sharma, S., Meehan, B. and Rak, J., 2019. Mapping subpopulations of cancer cell-derivedextracellular vesicles and particles by nano-flow cytometry. *ACS Nano, 13*(9), pp.10499–10511.
11. Dong, X., Niu, Y., Ding, Y., Wang, Y., Zhao, J., Leng, W. and Qin, L., 2017. Formulation and drug loading features of nano- erythrocytes. *Nanoscale Research Letters, 12*,p.202.
12. Natesan, S., Krishnaswami, V., Ponnusamy, C., Madiyalakan, M., Woo, T. and Palanisamy, R., 2017. Hypocrellin B and nano sil- ver loaded polymeric nanoparticles: Enhanced generation of singlet oxygen for improved photodynamic therapy. *Materials Science and Engineering: C, 77*,pp.935–946.
13. Liu, C., Shen, H., Wang, S., Cao, X., Xu, H., Xia, Y., Bai, T., Liu, Y., Peng, L., Li, C., Guo, Z. and Li, Z., 2018. Spermine increases bac- tericidal activity of silver-nanoparticles against clinical methicillin- resistant *Staphylococcus aureus. Chinese Chemical Letters, 29*(12), pp.1824–1828.
14. Yang, G., Xiao, Z., Tang, C., Deng, Y., Huang, H. and He, Z., 2019. Recent advances in biosensor for detection of lung cancer biomark- ers. *Biosensors and Bioelectronics, 141*,p.111416.
15. Wang,S.,Zhang,J.,Wang,C.,Yu,X.,Cai,W.andShao,X.,20 19.Determination of triglycerides in human serum by near-infrared dif- fuse reflectance spectroscopy using silver mirror as a substrate. *Chi- nese Chemical Letters, 30*(1), pp.111–114.
16. Deng, J., Ding, Q.M., Li, W., Wang, J.H., Liu, D.M., Zeng, X.X., Liu, X.Y., Ma, L., Deng, Y., Su, W. and Ye, B., 2020. Preparation of nano-silver-containing polyethylene composite film and Ag ion migration into food-simulants. *Journal Nanoscience and Nanotech- nology, 20*(3),pp.1613–1621.
17. Li, T.,Xiao,P.,Khan,A.,Wang,Z. andHe,N.,2017. PreparationofDNA-templated silver nanoclusters under macromolecular crowding conditions. *Nanoscience and Nanotechnology Letters, 9*(6), pp.892– 896.
18. Yang,G.,Lai,Y.,Xiao,Z.,Tang,C.andDeng,Y.,2018.Ultrase

n-sitive electrochemical immunosensor of carcinoembryonic antigen based on gold-label silver-stain signal amplification. *Chinese Chem- ical Letters, 29*(12), pp.1857–1860.

19. Li, T., Yi, H., Liu, Y., Wang, Z., Liu, S., He, N., Liu, H.andDeng, Y., 2018. One-step synthesis of DNA templated water-soluble Au–Ag bimetallic nanoclusters for ratiometric fluorescence detection of DNA. *Journal of Biomedical Nanotechnology, 14*(1),pp.150–160.
20. Li, T., He, N., Wang, J., Li, S., Deng, Y. and Wang, Z., 2016. Effects of the i-motif DNA loop on the fluorescence of silver nanoclus- ters. *RSC Advances, 6*(27),pp.22839–22844.
21. Li,T.,Yang,J.,Ali,Z.,Wang,Z.,Mou,X.,He,N.andWang,Z., 2017. Synthesis of aptamer-functionalized Ag nanoclusters for MCF-7 breast cancer cells imaging. *Science China Chemistry, 60*(3), pp.370–376.
22. Deng, J., Chen, Q.J., Peng, Z.Y., Wang, J.H., Li, W., Ding,Q.M.,Lin, Q.L., Liu, D.M., Wang, S.S., Shi, Y., Liu, Y. and Feng, S.S., 2019. Nano-silver-containing polyvinyl alcohol composite film for grape fresh-keeping. *Materials Express, 9*(9), pp.985–992.
23. Rao, K., Aziz, S., Roome, T., Razzak, A., Sikandar, B., Jamali, K.S., Imran,M.,Jabri, T.andShah,M.R.,2018.Gumacaciastabilizedsil- ver nanoparticles based nano-cargo for enhanced anti-arthritic poten- tials of hesperidin in adjuvant induced arthritic rats. *Artificial Cells, Nanomedicine, and Biotechnology, 46*(S1),pp.597–607.
24. Manresa, C., Sanz-Miralles, E.C., Twigg, J. and Bravo, M., 2018. Supportive periodontal therapy (SPT) for maintaining the dentition in adults treated for periodontitis. *The Cochrane Database of Sys- tematic Reviews, 1*(1),pp.1-50.
25. Joseph,B.,Janam,P.,Narayanan,S.andAnil,S.,2017.Isanti micro- bial photodynamic therapy effective as an adjunct to scaling and root planing in patients with chronic periodontitis? A systematic review. *Biomolecules, 7*(4),p.79.
26. Noronha, V.T., Paula, A.J., Durán, G., Galembeck, A.,Cogo-Müller, K., Franz-Montan, M. and Durán, N., 2017. Silver nanopar- ticles in dentistry. *Dental Materials, 33*(10),pp.1110–1126.
27. Kaczyński,T.,Wroński,J.,Gluszko,P.,Kryczka,T.,Miskiewicz, A., Górski, B., Radkowski, M., Strzemecki, D., Grieb, P. and Górska, R., 2019. Salivary interleukin 6, interleukin 8, interleukin 17A, and tumour necrosis factor alpha levels in patients with periodontitis and rheumatoid arthritis. *Central European Journal of Immunol- ogy, 44*(3),pp.269–276.
28. Brodzikowska, A., Górska, R. and Kowalski, J., 2019. Interleukin- 1 genotype in periodontitis. *Archivum Immunologiae et Therapiae Experimentalis, 67*(6),pp.367–373.
29. Bahammam, M.A. and Attia, M.S., 2018. Effects of systemic sim- vastatin on the concentrations of visfatin, tumor necrosis factor- alpha, and interleukin-6 in gingival crevicular fluid in patients with type 2 diabetes and chronic periodontitis. *Journal of Immunology Research, 2018*,p.8481735.
30. Islam, M.T., 2017. Oxidative stress and mitochondrial dysfunction- linked neurodegenerative disorders. *Neurological Research, 39*(1), pp.73–82.
31. Tsikas, D., 2017. Assessment of lipid peroxidation by measur- ing malondialdehyde (MDA) and relatives in biological samples: Analytical and biological challenges. *Analytical Biochemistry, 524*, pp.13–30.
32. Wang,Y.,Andrukhov,O.andRausch-Fan,X.,2017.Oxidati vestress and antioxidant system in periodontitis. *Frontiers in Physiology, 8*, p.910.
33. Tóthová, L. and Celec, P., 2017. Oxidative stress and antioxidants in the diagnosis and therapy of periodontitis. *Frontiers in Physiology, 8*, p.1055.
34. Honjoh, K., Nakajima, H., Hirai, T., Watanabe, S. and Matsumine, A., 2019. Relationship of inflammatory cytokinesfromM1-type microglia/macrophages at the injured site and lumbar enlargement with neuropathic pain after spinal cord injury in the CCL21 knockout (plt) mouse. *Frontiers in Cellular Neuro- science, 13*,p.525.
35. Hoter, A., El-Sabban, M.E. and Naim, H.Y., 2018. The HSP90 fam- ily: Structure, regulation, function, and implications in health and disease. *International Journal of Molecular Sciences, 19*(9),p.2560.
36. Ferreira, I.G., Carrascal, M., Mineiro, A.G., Bugalho, A., Borralho, P., Silva, Z., Dall'olio, F. and Videira, P.A., 2019. Carci- noembryonic antigen is a sialyl Lewis x/a carrier and an Eselectin ligand in nonsmall cell lung cancer. *International Journal of Oncol- ogy, 55*(5),pp.1033–1048.
37. Wang, T., Fu, J., Liu, X.Q. and Zhang, Q., 2020. Expression and sig- nificance of chemokines CCL21, E-selectins and Hsp90 in periodon- tal tissues of rats with experimental periodontitis. *Shanghai Journal of Stomatology, 29*(4),pp.350–354.
38. Sun, J., Li, S., Gao, Y., Zhang, P.P. and Yang, P.S., 2012. Estab- lishment of the inferior alveolar nerve transection combined exper- imental periodontitis animal modal in rats. *Shanghai Journal of Stomatology, 21*(1),pp.9–14.
39. Liao, C.H., Fei, W., Shen, Z.H., Yin, M.P. and Lu, C., 2014. Expres- sion and distribution of TNF-alpha and PGE2 of periodontal tis- sues in rat periodontitis model. *Asian Pacific Journal of Tropical Medicine, 7*(5),pp.412–416.
40. Hassumi, J.S., Mulinari-Santos, G., Fabris, A.L.D.S., Jacob, R.G.M., Gonçalves, A., Rossi, A.C., Freire, A.R., Faverani, L.P. and Okamoto, R., 2018. Alveolar bone healing in rats: Micro-CT, immunohistochemical and molecular analysis. *Journal of Applied Oral Science, 26*,p.e20170326.