

The Effect of Silicone Oil and Nano-hydroxyapatite/Chitosan Powder on Microhardness and Surface Structure of Primary Teeth Enamel After Iron Drop Consumption

¹Mitra Tabari ²Homayoun Alaghemand ³Mahmood Rabiee ⁴Sorayya Khefri *⁵Massoud Seyyed Ahadi ⁶Mohammad-Reza Nikpour

¹Assistant Professor, Dept. of Pediatric Dentistry, Dental Materials Research Center, School of Dentistry, Babol University of Medical Sciences, Babol, Iran.

²Associate Professor, Dept. of Operative Dentistry, Dental Materials Research Center, School of Dentistry, Babol University of Medical Sciences, Babol, Iran.

³Assistant Professor, Dept. of Metallurgy, Mechanic School, Babol University of Technology, Babol, Iran.

⁴Assistant Professor, Dept. of Biostatistics, School of Medicine, Babol University of Medical Sciences, Babol, Iran.

*⁵Assistant Professor, Dept. of Pediatric Dentistry, Ardebil University of Medical Sciences, Ardebil, Iran. E-mail: masud_us2002@yahoo.com

⁶Researcher, Nanobiotechnology Research Center, Babol University of Technology, Babol, Iran.

Abstract

Objective: Oral iron drops are recommended for children aged 6-24 months to prevent iron-deficiency anemia. However, their use is associated with the potential risk of erosion and microhardness reduction of primary teeth enamel due to their high acidity. It seems that the combination of silicone oil, nano-hydroxyapatite and chitosan powder may be able to improve the microhardness and surface structure of primary teeth enamel. This study sought to assess the effect of silicone oil plus nano-hydroxyapatite/chitosan on the changed microhardness and surface structure of primary teeth enamel after exposure to iron drop.

Methods: In this in-vitro study, 30 anterior deciduous teeth were collected and randomly divided into 3 groups. In the first group, samples were exposed to iron drop for 5min and then treated with nano-hydroxyapatite/chitosan and silicone oil for 10min. In the second group, specimens were first treated with the mentioned compound and then exposed to iron drop. Microhardness changes were compared in each group using Wilcoxon one sample or Sign test and between the two groups using Mann Whitney U test. The third group specimens were observed with a scanning electron microscope (SEM) to assess their surface texture and quality.

Results: In the first group, iron drop exposure decreased enamel microhardness by 44% of the baseline value ($p < 0.001$). Application of the understudy compound after iron drop exposure significantly increased enamel microhardness by 3% of the baseline value ($p = 0.006$). Use of the mentioned compound before iron drop exposure significantly increased enamel microhardness by 2% of the baseline value ($p = 0.023$). No statistically significant difference was found between the two groups ($p = 0.74$).

Conclusion: Iron drop exposure significantly decreased enamel microhardness. Use of silicone oil and nano-hydroxyapatite/chitosan powder caused 3% and 2% increase in enamel microhardness in the first and 2nd group, respectively. These values, compared to the effect of iron drop exposure are clinically significant.

Key words: Dental erosion, Iron drop, Microhardness, Nano-hydroxyapatite/chitosan, Primary teeth enamel, Silicone oil.

Please cite this article as follows:

Tabari M, Alaghemand H, Rabiee M, Khefri S, Seyyed Ahadi SM, Nikpour MR. Effect of silicon oil and nanohydroxy apatite/chitosan powder on microhardness and surface structure changes of primary teeth enamel after iron drop consumption. *J Dent Sch* 2013; 31(3):138-147.

Received: 28.08.2012

Final Revision: 14.05.2013

Accepted: 01.06.2013

Introduction:

Iron deficiency anemia is the most common type of childhood anemia. Iron drops are usually administered to prevent this condition (1). Prevention of iron deficiency anemia improves the capacity of learning and physical, mental and cognitive growth and development of infants and in long-term is a profitable investment for the countries (2). Iron drop contains citrate and has high acidity (3). Thus, it can cause erosion, decrease enamel strength and accelerate the process of caries development (4). Acidic content of one single dose of a drug may seem insignificant bit when it is used for weeks to months, it would be a serious threat to dental health (5). Limited studies have evaluated the adverse erosive effects of iron drop on deciduous teeth enamel.

Primary teeth enamel is thinner than the permanent teeth enamel. It is more fragile and softer than the permanent teeth enamel and thus is more susceptible to fracture. Primary enamel has higher organic content and lower microhardness than permanent enamel and the usual crystalline structure does not exist in the outermost surface of deciduous teeth enamel (6). Nano-hydroxyapatite particles have the highest resemblance to tooth enamel in terms of physicochemical, mechanical and biological characteristics and can remineralize the decalcified areas and repair the defective enamel (7). Nano-hydroxyapatite particles have been extensively studied in the recent years due to their unique remineralizing properties. It has been proven that they can protect enamel from demineralization and remineralize it (8-11).

Nano-hydroxyapatite is hydrophilic and has greater surface area than the conventional hydroxyapatite crystals. Thus, these crystals have high wettability and form a thin but strong layer on enamel surface that bonds to tooth structure (12).

Chitosan is a biopolymer derived from the

exoskeleton of crustaceans. It possesses antimicrobial, anti-plaque, salivary stimulant and enamel remineralizing properties (13). It also has many applications in medicine such as in wound healing (14), drug release and delivery systems (15-17), bioactive membranes (18), implant coating (19), bone tissue engineering (20, 21), blood vessel repair (22) and nerve repair (23, 24). Chitosan has bacteriostatic and bactericidal properties as well (25, 26). It is non-toxic and biodegradable (27, 28) and is also capable of improving and modifying the nano-hydroxyapatite surfaces (29).

Since hydroxyapatite is a favorable bioactive material and chitosan has optimal flexibility, together they can create a homogenous compound with higher strength and adhesion properties than pure hydroxyapatite (30, 31).

Silicone oil decreases the high surface energy of inter-crystalline spaces. This is particularly important in ion delivery into or out of the enamel especially in primary carious lesions. Also, if applied to tooth enamel, it decreases protein adsorption to hydroxyapatite and decelerates the process of pellicle formation on the enamel surface (32, 34). This study sought to assess the effect of nano-hydroxyapatite/chitosan plus silicone oil on microhardness and surface structure of primary teeth enamel after exposure to iron drop.

Methods:

In this in-vitro study, 30 anterior deciduous teeth (A, B and C) were selected using convenience sampling. The teeth had no caries, cracks or hypocalcification and had been extracted less than 3 months ago for orthodontic purposes, space shortage or mobility. The teeth were stored in 0.9% saline solution (Shahid Ghazi Pharmaceuticals, Tabriz) at room temperature until the conduction of study. The solution was refreshed daily. The teeth were not treated with any disinfectant agent because chemical agents

can affect enamel microhardness. Nano-hydroxyapatite/chitosan powder was produced in Babol School of Technology, Nano-Research Center using in situ hybridization method. First, 4g chitosan obtained from Fluka (Sigma-Aldrich, Munich, Germany) was dissolved in 100 cc water and 2% acetic acid (Merck, Darmstadt, Germany) and mixed with an electric mixer for 24h. Next, 100 cc of 2 molar $\text{CaNO}_3(\text{H}_2\text{O})_4$ (Merck, Darmstadt, Germany) was added. To control the growth of particles, polyethylene glycol (Merck, Darmstadt, Germany) was used (solution A). After 4 hours of high-power mixing, 100cc of 2 molar

diammonium hydrogen phosphate solution (Merck, Darmstadt, Germany) along with 1g cetyltrimethyl bromide (Merck, Darmstadt, Germany) were gradually added to the solution A. simultaneously, titration was done by adding sodium hydroxide (Merck, Darmstadt, Germany) to maintain the pH of the system in the range of 11-12. The final solution was mixed for 24h to yield a homogenous suspension. The final product was then filtered by centrifugation at 3000 rpm and converted to a white powder by freeze-drying at -50°C . Ferrous sulfate iron drop (Kharazmi Co., Iran) with the following characteristics was used in this study.

Table 1- Characteristics of the used iron drop

Iron drop	Manufaturing country	pH	TA to pH 7	PO4 mmol/l	Ca mmol/l	Floride Ppm	Sweetener	Amount of effective material in 1 mg of drug	Citrate ppm
$\text{Fe}(\text{SO}_4)(\text{H}_2\text{O})_7$ 125 mg	Iran	2.1	2.37	119.87	0.375	0.01	Saccharin	25 mg iron ion	102

On the buccal surface of teeth, a window measuring 4x4 mm was marked with a label and its surrounding area was covered with clear nail varnish. The label was then removed and the excessive glue was washed off by distilled water. This was done to match the test area in teeth. To increase the adhesion of adhesive material, the slide surface was sandblasted. The teeth were then bonded to the microscope slide using an adhesive agent. The teeth were mounted in order to be fixed for polishing and observation under a microscope. To exert the effect of iron drop, the apices were obstructed by adhesive wax. Since the intact surface enamel has higher fluoride content and is more acid-resistant than the underlying layers (35), we tried to minimize the labial surface polishing by using 6000 grit silicon carbide papers under running water. We minimally polished enamel surfaces because we wanted to preserve the outermost enamel surface in order to simulate oral environment. We only smoothed the surface to the level that we could symmetrically create indentation. To simulate clinical setting, the iron

drop used had a temperature of 9°C similar to the temperature of a refrigerated iron drop. To dilute the iron drop, artificial saliva was added to it (1.5 times its volume) and the total volume was reached to the level where the teeth could be easily immersed in it. The nano-hydroxyapatite/chitosan powder and the silicone oil carrier were experimentally (powder to liquid ratio of 1 to 3) mixed and prepared in a way that it was applicable and adhesive to tooth structure. All 30 teeth were immersed in artificial saliva (Kin Laboratories, Barcelona, Spain) in an incubator shaker (HeidolphUnimax 1010, Germany) at 37° for an hour before testing to simulate oral environment and were then subjected to Vickers microhardness test using Koopa MH1 microhardness tester (Koopa Pajouhesh, Sari, Iran). The load was automatically applied by the indenter to the respective point with a feedback with 1g precision. In this study, we used 50g load for 10s to measure the microhardness of specimens. After load application, by adjusting the microscope, length of the diagonal left by the indenter was read and entered the control device

to calculate hardness. For each specimen, hardness, length of the diagonal left by the indenter and penetration depth were recorded. Microhardness was calculated for each sample based on the distance of 3 indentations on the smooth surface on each tooth and the mean of 3

values was calculated and reported in VHN. Teeth with 239-478 HV (hardness Vickers) were selected. The excluded teeth were replaced with teeth with the same range of microhardness. Of 30 teeth, 20 were randomly selected and divided into 2 groups.

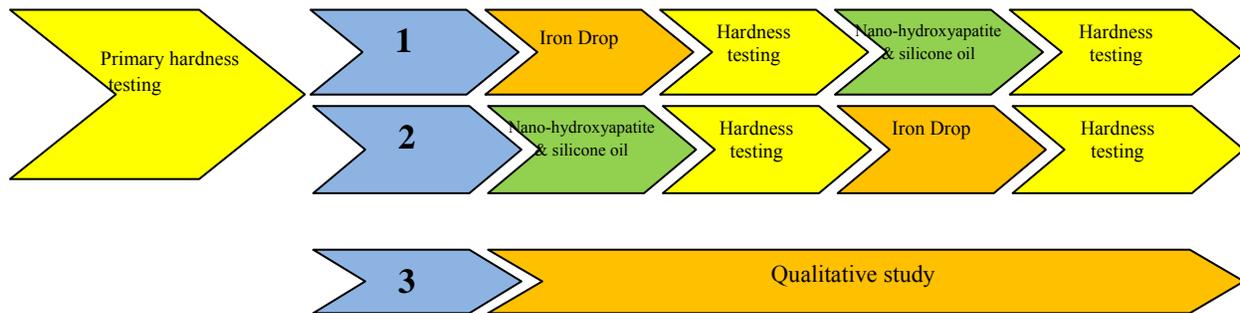


Diagram 1- Classification of samples and the 2 understudy methods

In group 1, the teeth were immersed in iron drop for 5min in an incubator shaker (HeidolphUnimax 1010, Schwabach, Germany), rinsed with distilled water and subjected to microhardness testing. Next, nano-hydroxyapatite/chitosan plus silicone oil was applied to tooth surface with an applicator and the teeth were subjected to microhardness testing again.

In group 2, nano-hydroxyapatite/chitosan plus silicone oil was applied to tooth surfaces with an applicator for 10 min and then the teeth were subjected to hardness testing. Specimens were then immersed in iron drop solution for 5 min and underwent microhardness testing again. Microhardness was measured by an operator blinded to the group allocation of specimens. The remaining 10 teeth were used to qualitatively assess enamel surfaces at different phases. Of the mentioned 10 teeth, 2 remained intact and 2 after application of iron drop, 2 after application of nano-hydroxyapatite/chitosan +silicone oil and then iron drop and 2 after application of iron drop followed by the mentioned compound were prepared as described earlier and sent to the laboratory for

SEM analysis. Two specimens were allocated for SEM analysis at each step so that the second specimen could be used if the SEM image of the first one could not be obtained successfully. In the lab, the teeth were vacuumed, dehydrated, gold coated (Baltec, Switzerland) and subjected to SEM analysis (SEMKYKY-EM3200-2011, Beijing, China).

SPSS version 15 software was used for data analysis. Microhardness changes were evaluated in each group using Wilcoxon one sample or sign test and between groups using Mann Whitney U test. $p < 0.05$ was considered statistically significant.

Results:

In the first group, after 5 min of exposure to iron drop, the tooth surface was treated with nano-hydroxyapatite/chitosan plus silicone oil for 10min while in the second group, the teeth were first treated with the mentioned compound and then exposed to iron drop.

Method one:

Exposure to Kharazmi iron drop decreased the baseline microhardness of deciduous teeth by 44%. The mean enamel microhardness

decreased from 351(55.94) to 195 (64.16). The amount of change was 155.29 (22).89 which was statistically significant ($p<0.001$).

Use of the understudy product after iron drop exposure increased enamel microhardness by 3% of the baseline value. The mean microhardness increased from 195 (64.16) to 207 (61.07). The mean amount of change was 11.50 (10.22) and this change was statistically significant ($p=0.006$).

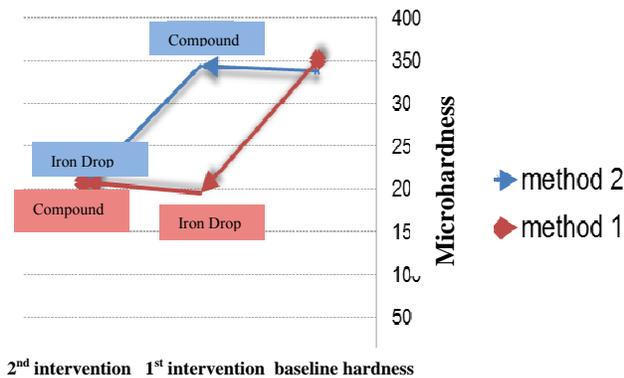


Diagram 1- Comparison of change in microhardness of enamel between the two methods

Method two:

Use of the understudy compound before iron drop exposure increased enamel microhardness by 2% of the baseline value. The mean enamel microhardness increased from 337 (60.68) to 344 (57.27). The amount of increase was 6.80 (7.88); which was statistically significant ($p=0.023$). Exposure to Kharazmi iron drop after the use of mentioned compound decreased the baseline microhardness of enamel by 40% of the baseline value. The mean microhardness decreased from 344 (57.27) to 203 (55.81). The amount of reduction was 141.50 (12.86); which was statistically significant ($p<0.001$).

Comparison of the two methods:

The difference between the effects of two methods on enamel microhardness was not significant ($p=0.74$).

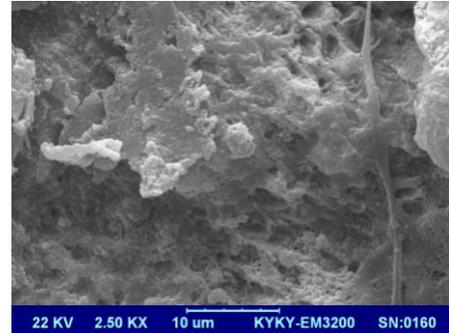


Figure 1- SEM image of enamel surface shows enamel porosities caused by the dissolution of enamel crystals after exposure to iron drop

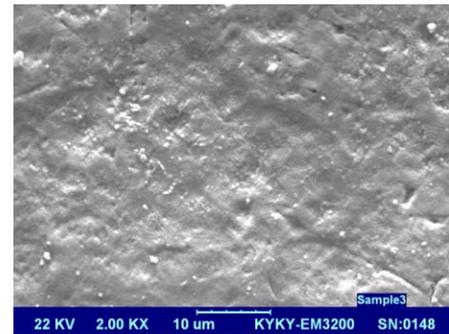


Figure 2- SEM image of enamel surface after treatment with nano-hydroxyapatite/chitosan+silicone oil following iron drop exposure: the porosities are covered with the compound

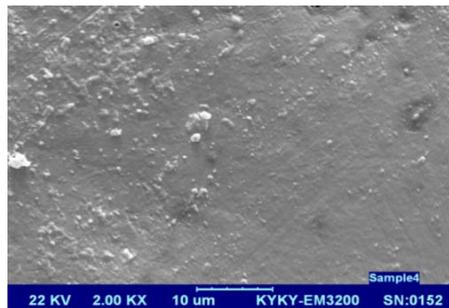


Figure 3- SEM image of enamel surface after treatment with nano-hydroxyapatite/chitosan+silicone oil and subsequent exposure to iron drop: the understudy material prevented the destructive effects of iron drop to some extent

Discussion:

Exposure to iron drop significantly decreases enamel microhardness. Use of nano-hydroxyapatite/chitosan powder plus silicone oil improved enamel microhardness by 3% and 2% in the first and second groups, respectively.

These amounts are clinically significant.

Limited studies have evaluated the potential effects of iron drop on deciduous teeth enamel (36-38). James and Parfitt in 1953 confirmed the potentially erosive effects of iron drops (both Iranian and foreign made). They demonstrated that the erosive potential of Kharazmi iron drop was greater than that of Ironorm iron drop (3).

In our study, exposure to iron drop decreased the deciduous teeth enamel microhardness. The amount of reduction in microhardness in our study was similar to that in James and Parfitt's study (1953). This similarity may be explained by the similar exposure time of teeth to iron drop; which was 5 min in both studies (3). The pH of iron drop in our study was 2.1; which is below the critical pH of enamel. As observed in SEM images, extensive destruction of enamel crystalline structure due to exposure to iron drop indicates the erosive nature of acid in its composition. However, duration and method of exposure to iron drop require further investigations. In order to simulate oral environment of a child, the teeth should be exposed to iron drop repeatedly with shorter durations over a longer time period. In this study, iron drop was diluted with artificial saliva to simulate clinical setting. However, it should be noted that the erosive capacity of acidic beverages is not due to their low pH alone and is greatly influenced by their mineral content, buffering capacity and decalcifying properties (39). Lussi and Jaeggi in 2006 stated that yogurt with a pH of 1.4 has no erosive capacity because of its high calcium and phosphate content (40). Kharazmi iron drop has very low calcium and phosphate content and a very low pH as well. Thus, it has a high destructive power.

Drinking method of acidic beverages also plays a role in pH drop and increased demineralization. The faster a liquid is washed off the mouth the lower its destructive effects (41).

Previous studies have confirmed that size of

calcium phosphate crystals plays an important role in hard tissue formation and has a significant effect on its solubility and biocompatibility (42, 43). Well-sized nano-hydroxyapatite particles in comparison with conventional hydroxyapatite crystals (with hundreds of nm length) have greater potential to remineralize primary erosive enamel lesions (10). The size of nano-hydroxyapatite crystals in our study was in the range of 42-200 nm (44). PH of the environment also affects the physicochemical characteristics of nano-hydroxyapatite particles (45). Studies have reported that in pH of less than 7, the remineralizing effects of nano-hydroxyapatite particles significantly increased. Due to several reasons, in more acidic environments, nano-hydroxyapatite particles are more capable of remineralizing the tooth surface (46). Some of these reasons are as follows:

1. Acidic environments increase the solubility of nano-hydroxyapatite particles. Thus, the nano-hydroxyapatite solution is saturated and better deposited on the demineralized areas (46).
2. The electrostatic force between the nano-hydroxyapatite particles and the tooth enamel is decreased and nano-hydroxyapatite particles better deposit on tooth enamel (47-49).
3. Under these circumstances, higher concentrations of calcium and phosphate are released and remineralize the demineralized areas. Furthermore, in acidic solutions, mineral ions have higher penetration compared to a neutral remineralizing solution (50).

Chitosan functions better in the acidic environments as well because the chitosan amino groups absorb the hydrogen ions and become positively charged and capable of adhering to surfaces with a negative charge such as the tooth enamel, soft tissue and cell membrane (51, 52). Thus, it seems that nano-

hydroxyapatite/chitosan powder plus silicone oil would be more effective if applied after exposure to iron drop due to the presence of acidic environment. However, our study did not yield such result.

Similar studies have shown that nano-hydroxyapatite has remineralizing properties when used in mouth rinses, toothpastes and chewing gums. However, in all of these studies, nano-hydroxyapatite was combined with another compound with a possible synergistic effect. For instance, Huang in his study in 2009 used fluoride combined with nano-hydroxyapatite (7). In our study, nano-hydroxyapatite plus silicone oil was prepared in different ratios and experimentally examined to obtain a concentration that could be adhered to tooth surface. Eventually, 3 to 1 volume ratio was selected for application on the tooth surface. However, further investigations are required in this respect.

In our study, enamel microhardness increased by 3% when the mentioned compound was applied after exposure to iron drop. This amount was 2% when the mentioned compound was used before iron drop exposure. These rates were higher than the rate reported by Haghgoo *et al.* in 2011. They used pure nano-hydroxyapatite and reported a 1.5% increase in enamel microhardness. Since the study conditions were similar, this difference may be attributed to the presence of chitosan and its effective role in remineralization (53).

Researchers have reported different time periods for the application of this material (7, 31, 54-56). It has been shown that 10 min time had no significant difference with longer time periods in terms of the effectiveness of nano-hydroxyapatite for enamel remineralization. Roveri *et al.* in 2008 and Haghgoo *et al.* in 2011 also used 10min time in their studies (9, 53). Thus, we selected 10 min time period to simulate clinical setting. We assumed that most patients would be able to hold this product for

10 min in their mouth.

In previous studies on the effect of chitosan on enamel remineralization alone, amongst 30, 60 and 90s exposure times, 60s exposure time had the highest efficacy for remineralization (57).

Overall, it should be noted that in the process of erosion, the teeth are subjected to strong acidic attacks and thus, demineralization due to acid attack is much greater than that in the process of caries development. It means that remineralization mechanisms in erosion may not be as effective as in caries development (58).

Based on the obtained results, nano-hydroxyapatite/chitosan powder plus silicone oil had small remineralizing effect on deciduous teeth enamel when used before or after iron drop exposure and the erosive potential of iron drop was much greater than the remineralizing effect of the understudy compound.

To obtain more reliable results, in-vivo studies should be carried out. Furthermore, since chitosan was also incorporated into the understudy compound, further studies are required to determine the best duration of usage for the best possible results.

Conclusion:

Exposure to iron drop significantly decreased enamel microhardness. Application of nano-hydroxyapatite/chitosan powder plus silicone oil increased enamel microhardness by 3% and 2% in the first and second groups, respectively. Compared to enamel microhardness reduction due to exposure to iron drop, these amounts are clinically significant.

Acknowledgement

The authors would like to thank all instructors at Babol University of Medical Sciences and the personnel of Dental Materials Research Center of Babol School of Dentistry.

Conflict of Interest: “None Declared”**References:**

1. Demaeyer E, Adiels-Tegman M. The prevalence of anemia in the world. *World Health Stat Q* 1985; 38: 302-316.
2. FesharakiNia A, SharifZadeh GhR. Effective factors on mothers' performance regarding supplementary iron-drop taking by their children in Birjand [Persian]. *J Birjand Uni Med Sci* 2006; 13: 9-15.
3. James PMC, Parfitt GJ. Local effects of certain medicaments on the teeth. *Br Med J.* 1953; 2: 1252-1253.
4. Gilchrist F, Santini A, Harley K, Deery C. The use of micro-Ramanspectroscopy to differentiate between sound and eroded primary enamel. *Int J Pediatr Dent* 2007; 17: 274-280.
5. Nunn JH, Ng SK, Sharkey I, Coulthard M. The dental implications of chronic use of acidic medicines in medically compromised children. *Pharm World Sci* 2001; 23:118-119.
6. Low I M, Duraman N, Fulton J, Tezuka N, Davies IJ. A comparative study of the microstructure property relationship in human adult and baby teeth. *Ceram Eng Sci Proceed* 2005; 26:145-152
7. Huang SB, Gao SS, Yu HY. Effect of nano-hydroxyapatite concentration on remineralization of initial enamel lesion in vitro. *Biomed Mater* 2009; 4: 034104.
8. Hannig M, Hannig C. Nanomaterials in preventive dentistry. *Nat Nanotechnol* 2010; 5: 565– 569.
9. Roveri N, Battistella E, Foltran I, Foresti, E, Iafisco M, Lelli M, *et al.* Synthetic biomimetic carbonate-hydroxyapatite nanocrystals for enamel remineralization. *Adv Mater Res* 2008; 47: 821-824.
10. Li L, Pan HH, Tao JH, Xu XR, Mao CY, Gu XH, *et al.* Repair of enamel by using hydroxyapatite nanoparticles as the building blocks. *J Mater Chem* 2008; 18: 4079-4084.
11. Lv KL, Zhang JX, Meng XC, Li XY. Remineralization effect of the nano-HA toothpaste on artificial caries. *Key Eng Mater* 2007; 330-332: 267-270.
12. Porsgaard TK, Gyldenvang L. Chewing gum comprising hydroxyapatite. *Fresh Patents* 2009; 1:424-448
13. Hayashi Y, Ohara N, Ganno T, Yamaguchi K, Ishizaki T, Nakamura T, *et al.* Chewing chitosan containing gum effectively inhibits the growth of cariogenic bacteria. *Arch Oral Biol* 2007; 52: 290-294.
14. Khor E, Lim LY. Implantable applications of chitin and chitosan. *Biomaterials* 2003; 24: 232339-232349.
15. Han DK, Park KD, Hubbell JA, Kim YH. Surface characteristics and biocompatibility of lactide-based poly (ethylene glycol) scaffolds for tissue engineering. *J Biomater Sci Polym Ed* 1998; 9: 667-680.
16. Vunjak-Novakovic G, Obradovic B, Martin I, Bursac PM, Langer R, Freed LE. Dynamic cell seeding of polymer scaffolds for cartilage tissue engineering. *Biotechnol Prog* 1998; 14: 193-202.
17. Yannas IV. Applications of ECM analogs in surgery. *J Cell Biochem* 1994; 56:188-191.
18. Bumgardner JD, Wisner R, Gerard PD, Bergin P, Chestnutt B, Marin M, *et al.* Chitosan: potential use as a bioactive coating for orthopaedic and craniofacial/dental implants. *J Biomater Sci Polym Ed* 2003; 14: 423-438.
19. Schakenraad JM, Busscher HJ, Wildevuur CR, Arends J. The influence of substratum surface free

- energy on growth and spreading of human fibroblasts in the presence and absence of serum proteins. *J Biomed Mater Res* 1986; 20: 773-784
20. Kong L, Gao Y, Cao W, Gong Y, Zhao N, Zhang X. Preparation and characterization of nano-hydroxyapatite/ chitosan composite scaffolds. *J Biomed Mater Res* 2005; 75: 275-282.
 21. Zhao F, Grayson WL, Ma T, Bunnell B, Lu WW. Effects of hydroxyapatite in 3-D chitosan-gelatin polymer network on human mesenchymal stem cell construct development. *Biomater* 2006; 27: 1859-1867.
 22. Zhang L, Ao Q, Wang A, Lu G, Kong L, Gong Y, *et al.* A sandwich tubular scaffold derived from chitosan for blood vessel tissue engineering. *J Biomed Mater Res* 2006; 77: 277-284.
 23. Ao Q, Wang A, Cao W, Zhang L, Kong L, He Q, *et al.* Manufacture of multimicrotubule chitosan nerve conduits with novel molds and characterization in vitro. *J. Biomed Mater Res A.* 2006, 77, 8-11.
 24. Cao W, Cheng M, Ao Q, Gong Y, Zhao N, Zhang X. Physical, mechanical and degradation properties, and Schwann cell affinity of cross-linked chitosan films. *J Biomater Sci Polym Ed* 2005; 16:791-807.
 25. Kong M, Chen XG, Liu CS, Liu CG, Meng XH, Yu LeJ. Antibacterial mechanism of chitosan microspheres in a solid dispersing system against *E coli*. *Colloids Surf B Biointerfaces* 2008; 65: 197-202.
 26. Fujiwara M, Hayashi Y, Ohara N. Inhibitory effect of water-soluble chitosan on growth of *Streptococcus mutans*. *New Microbiol* 2004; 27:83-86.
 27. Tharanathan RN, Kittur FS. Chitin-the undisputed biomolecule of great potential. *Crit Rev Food Sci Nutr* 2003; 43: 61-87.
 28. Rinaudo M. Chitin and chitosan: properties and applications. *Prog Polym Sci* 2006; 31:603–632.
 29. Wilson OC Jr, Hull JR. Surface modification of nanophase hydroxyapatite with chitosan. *Mater Sci Eng C* 2008; 28:434-437.
 30. Thein-Han WW, Misra RD. Biomimetic chitosan–nanohydroxyapatite composite scaffolds for bone tissue engineering. *Acta Biomater* 2009; 5: 1182-1197.
 31. Kim MY, Kwon HK, Choi CH, Kim BI. Combined effects of nano-hydroxyapatite and NaF on remineralization of early caries lesion. *Key Eng Mater* 2007; 330–332: 1347-1350.
 32. Boyers CL, Shaw JH, Rosenthal E, Giordano R. Effects of silicone oil on the teeth of caries-susceptible rats when applied by tooth brushing and by inclusion in the diet. *J Dent Res* 1963; 42: 1517-1519.
 33. Rølla G, Ellingsen JE, Gaare D. Polydimethylsiloxane as a tooth surface-bound carrier of triclosan: a new concept in chemical plaque inhibition. *Adv Dent Res* 1994; 8: 272-277.
 34. Rykke M, Rølla G. Effect of silicone oil on protein adsorption to hydroxyapatite in vitro and on pellicle formation in vivo. *Scand J Dent Res* 1990; 98: 401-411.
 35. Eskandarian T, Joshan MJ. Evaluation of the dmft Index and Its relationship to some factors consisting the consumption of Iron supplementary drug in 2-5 years old kingergarden children in Shiraz. *Shiraz Univ Dent J* 2006; 6:1-9.
 36. Miguel JC, Bowen WH, Pearson SK. Effects of iron salts in sucrose on dental caries and plaque in rats. *Arch Oral Biol* 1997; 42: 377-383.
 37. Miguel JC, Bowen WH, Pearson SK. Effect of frequency of exposure to iron-sucrose on the incidence of dental caries in desalivated rats. *Caries Res* 1997; 31: 238-243.
 38. Rosalen PL, Pearson SK, Bowen WH. Effects of copper, iron and fluoride co-crystallized with

- sugar on caries development and acid formation in desalinated rats. *Arch Oral Biol* 1996; 41: 1003-1010.
39. Donovan T, Swift EJ Jr. Dental erosion. *J Esthet Restor Dent* 2009; 21: 359-364.
 40. Lussi A, Jaeggi T. Extrinsic causes of erosion. In Lussi A. *Dental erosion: from diagnosis to therapy*, 1st Ed. Switzerland, Basel: Karger; 2006; Chap 7: 77-87.
 41. Johansson AK, Lingström P, Imfeld T, Birkhed D. Influence of drinking method on tooth-surface pH in relation to dental erosion. *Eur J Oral Sci* 2004; 112: 484-489.
 42. Balasundaram G, Sato M, Webster TJ. Using hydroxyapatite nanoparticles and decreased crystallinity to promote osteoblast adhesion similar to functionalizing with RGD. *Biomaterials* 2006; 27: 2798-2805.
 43. Hu Q, Tan Z, Liu Y, Tao J, Cai Y, Zhang M, *et al.* Effect of crystallinity of calcium phosphate nanoparticles on adhesion, proliferation, and differentiation of bone marrow mesenchymal stem cells. *J Mater Chem* 2007; 17: 4690-4698.
 44. Nikpour MR, Rabiee MR, Jahanshahi M. Synthesis and characterization of hydroxyapatite/chitosan nanocomposite materials for medical engineering applications. *Composites Part B: Engineering* 2012; 43:1881-1886.
 45. Harding IS, Rashid N, Hing KA. Surface charge and the effect of excess calcium ions on the hydroxyapatite surface. *Biomaterials* 2005; 26: 6818-6826.
 46. Huang S, Gao L, Cheng L, Yu H. Remineralization potential of nano-hydroxyapatite on initial enamel lesions: an in vitro study. *Caries Res* 2011; 45: 460-468.
 47. Yin G, Liu Z, Zhan J, Ding F, Yuan N. Impacts of the surface charge property on protein adsorption on hydroxyapatite. *Chem Eng J* 2002; 87: 181-186.
 48. Skartsila K, Spanos N. Surface characterization of hydroxyapatite: potentiometric titrations coupled with solubility measurements. *J Colloid Inter face Sci* 2007; 308: 405-412.
 49. Huang LZ, Tang L, Xu R. [A bio electrochemical theory in pathogenesis of dental caries]. *Zhonghua Kou Qiang Yi Xue Za Zhi* 1997; 32: 34-36. [Article in Chinese]
 50. Yamazaki H, Margolis HC. Enhanced enamel remineralization under acidic conditions in vitro. *J Dent Res* 2008; 87: 569-574.
 51. Sano H, Shibasaki R, Matsukubo T, Takaesu Y. Effect of chitosan rinsing on reduction of dental plaque formation. *Bull Tokyo Dent Coll* 2003; 44: 9-16.
 52. Sezer AD, Cevher E, Hatipoğlu F, Oğurtan Z, Bas AL, Akbuğa J. Preparation of fucoidan-chitosan hydrogel and its application as burn healing accelerator on rabbits. *Biol Pharm Bull* 2008;31:2326-2333.
 53. Haghgoo R, Abbasi F, Rezvani MB. Evaluation of the effect of nanohydroxyapatite on erosive lesions of the enamel of permanent teeth following exposure to soft beer (in vitro). *J Dent Sch, Shahid Beheshti* 2011; 29: 149-154.
 54. Meng XC, Li XY, Lu KL. Study of Nano- Hydroxyapatite on the remineralization of Dismineralized teeth. *Mater. Sci. Forum* 2005;457: 2423-2426.
 55. Jeong SH, Jang SO, Kim KN, Kwon HK, Park YD, Kim BI. Remineralization potential of new toothpaste containing nanohydroxyapatite. *Key Engin Mat* 2006; 309-311: 537-540.
 56. Arnaud TM, de Barros Neto B, Diniz FB. Chitosan effect on dental enamel de-remineralization .an in vitro evaluation. *J Dent* 2010; 38:848-852.
 57. Chunmuang S, Jitpukdeebodindra S, Chuenarrom C, Benjakul P. Effect of xylitol and fluoride on enamel erosion in vitro. *J Oral Sci* 2007; 49:293-297.