ASDJ
AINSHAMS DENTAL JOURNAL
Print ISSN 1110-7642
Online ISSN 2735-5039

AIN SHAMS DENTAL JOURNAL
Official Publication of Ain Shams Dental School
June 2025 • Vol. 38

Effect of Addition of Omega-3 Fatty Acids to Nano-Hydroxyapatite on its Physical Properties: An In-Vitro Study

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Aim: This study evaluated the physical properties of nano-hydroxyapatite (nHA) after the addition of Omega-3 (ω3) powder in terms of radiopacity, solubility & pH.
Materials and methods: Radiopacity of group I (nHA), group II (2nHA:1ω3), & group III (1nHA:1ω3) were digitally evaluated against aluminium step wedge, human dentin & bovine cortical bone. Solubility of Calcium (Ca^{2+}) was evaluated after 1,3 & 5 days in Tris-HCL solution of 7.4 pH & Citric Acid solution after 5 days using atomic absorption spectroscopy. pH was evaluated along with the solubility test in Tris-HCL at 0h, 1, 3 & 5 days.
Results: The highest radiopacity level was recorded in nHA group. While groups containing ω3 showed the least mean grey value. Solubility of Ca^{2+} ions increased with the increase in immersion time & with the decrease in the pH of the solution. In both solutions, group III showed the greatest mean of Ca^{2+} concentration released followed by group II & group I during all observation periods. In Tris-HCL, initial rise in pH up to 7.94 was observed in group I at 0h followed by a gradual drop reaching a pH value of 6.72 at day 5. While in groups II & III, pH showed an initial decrease at 0h, a gradual increase followed from day 1 to 5.
Conclusion: Adding (ω3) to (nHA) lowered its radiopacity and increased its solubility in Tris-HCL & acidic medium. (nHA) calcium release was not affected by low pH, offering a clinical advantage in case of bacterial infection.

Keywords: Nano-Hydroxyapatite, Omega-3, Radiopacity, Calcium Solubility, pH.

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Introduction

The need to restore large bone defects with suitable graft material is of great importance. Despite being the gold standard, autografts may present inconveniences limiting their use regarding harvesting, morbidity at the donor site & inadequate quantity.[1,2,3] Also, allografts & xenografts may elicit graft rejection, transfer of infection & immunological responses.[3] In an attempt to develop a non-immunogenic bone repair material that has the same structure as natural bone & with no disease transmission risks; synthetic biomaterials were introduced.[4]

Nano-Hydroxyapatite is an alloplastic bone graft with favorable physicochemical & biological properties. It is chemically resembling bone, in addition to being bioactive, biocompatible, & osteoconductive. It also has an increased surface area, & an increased surface roughness (for better cellular adhesion & host-tissue interaction).[1,3,5,6]

Furthermore, Nano-Hydroxyapatite enhances the adhesion & differentiation of human mesenchymal stem cells & osteoblasts, while decreasing the adhesion of fibroblasts.[7,8] It also enhances the proliferation of bone marrow stem cells and stimulates endothelial cells to promote angiogenesis.[9,10]

Although nHA had better solubility than its micro-sized counterpart, it was reported that it has slower solubility than other grafts which might have a negative impact on bone defect repair.[4,11]

Omega-3 is one of the major classes of polyunsaturated fatty Acids (PUFA). Several different forms of Omega-3 exist mainly as Alpha-Linolenic Acid (ALA) which is found predominantly in plant sources, Eicosapentaenoic acid (EPA), & Docosahexaenoic acid (DHA) are predominantly found in animal products such as fish, & seafood.[12]

Studies proposed that omega-3 can be used as an adjuvant therapy to help reduce inflammation associated with cardiovascular disease, insulin resistance, rheumatoid arthritis & periodontitis due to their anti-inflammatory properties.[12,13]

It has been suggested that Omega-3 has a positive effect in downregulating inflammatory cell infiltration & has a beneficial effect on bone health, decreasing bone resorption & promoting bone deposition.[12,13,14,15] It acts by inhibiting the production of arachidonic acid metabolites & modulating lymphocyte proliferation.[16,17] Attenuation of inflammation is also achieved by omega 3 metabolites which leads to the synthesis of specialized pro-resolving lipid mediators (SPMs) such as Resolvins. SPMs are anti-inflammatory mediators, with the potential of inflammatory resolving capability, & immunoregulatory actions such as inhibiting trans-endothelial migration of neutrophils, thus preventing the infiltration of more inflammatory cells into sites of inflammation, and blocking proinflammatory cytokine production as TNFα, IL-1β.[17,18,19]

However, the effect of locally applying omega-3 on bone healing remains unclear & requires further investigation. Hence, the current study aims to test the effect of adding omega-3 fatty acids to nano-hydroxyapatite on the physical properties of the latter mentioned material.

Materials and Methods

Ethical approval

This study was conducted after the approval of the Research Ethics Committee of the Faculty of Dentistry at Ain Shams University in Egypt (FD ASU- REC/ ID 09171).

Materials

- **Nano-Hydroxyapatite (nHA)** (NanoTech Egypt for Photo-Electronics, Al Giza, Egypt) a white rod-like-shaped hydroxyapatite nanopowder of 100 ± 30 nm (L), & 20 ± 5 nm (D) & Ca/P ratio of 1.6.
- **Omega-3 powder (ω-3)** (Nutri Vita shop, USA) is the powder form of Omega-3 polyunsaturated fatty acids of fish oil origin with a high content of Docosahexaenoic Acid (DHA) (56.26 %) & Eicosapentaenoic Acid (EPA) (82%).

Methods

Radiopacity, solubility, & pH were tested for the following groups:

- **Group I:** Nano-Hydroxyapatite (nHA).
- **Group II:** Nano-Hydroxyapatite + Omega-3 (2% nHA:1% ω3). Prepared by weighing 1 mg of omega 3 powder & 2 mg of nano-hydroxyapatite powder using an electronic analytical balance with accuracy 10^{-4} (0.0001g) (Sartorius Secura224-1S Analytical, Germany.), then both powders were mixed manually until homogenous.
- **Group III:** Nano-Hydroxyapatite + Omega-3 (1% nHA: 1% ω3), prepared in the same manner as group II, except in mixing equal amounts of omega 3 powder & nano-hydroxyapatite powder.

Radiopacity test

This test was conducted in accordance with ISO 13116:2014(en).[20] In this study, in order to clearly distinguish between the tested materials & the surrounding tissues in clinical situations; dentin of freshly extracted human tooth (HD), bovine mandibular cortical bone (BC) & an aluminum (Al) step wedge were used as references.[21,22]

Specimens' preparation

a) Pellets (n=8) measuring 13mm in diameter & 1mm in thickness were manipulated by pressing the powder of each group in a 13mm mold at 5 tons for 5 min using manual pellet pressing machine *(Carver Inc, USA)*, without using a binder to avoid the any added effect on radiopacity measurements.[23] Then, pellets were stored at 37°C & 100% humidity for 48 hours to reduce their brittleness. The pellets' thickness was confirmed by a gauge micrometer (Figure 1)

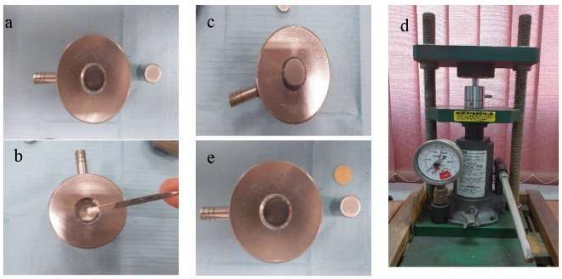

Figure1: Specimens Preparation: a. Die used to prepare pellets, b. Powder placed in a die, c. Closed Die, d. Closed Die placed in pellet press, e. Pellet prepared

b) Dentin (HD) specimens (n=8) were obtained by slicing freshly sound-extracted human teeth, in a longitudinal direction producing 1mm thick samples which were stored in purified water at 37°C till utilized.[21,24]

c) Bovine mandibular cortical bone (BC) specimens (n=8) were obtained from the mandible of a freshly sacrificed animal. Disc-shaped cortical bone specimens were prepared, each 1mm in thickness & approximately 1mm in diameter.[24]

Each specimen was digitally radiographed on a size 1.5 intraoral sensor *(EzSensor HD, Vatec, Korea.)* next to an aluminum (Al) step wedge near the center of the sensor using an X-ray machine (*Xgenus, De GÖtzen, Italia.)* operating at 70kV, 8mA, 0.320 exposure time, focal spot of 0.7mm, 30cm focus sensor distance & at 90° angle directed central X-ray beam. The aluminum step wedge was of 98% purity, with ten steps equally spaced, & thickness ranging from 1 to 10mm thickness. Digital images were exported to the Image J grey scale analysis software (National Institutes of Health, Bethesda, MD).[23,25]

Initially, a square area of (50×50 pixels) was defined as a region of interest (ROI) on the resulting images & for each step of the Al step wedge. The mean grey value (MGV) [range 0-255] was then measured & converted to mm Al.[23,25] The radiographic analysis was repeated three times.

Solubility tests

Tests were conducted in accordance with the procedures outlined in ISO 10993-14:2001.[26] This test involved two parts: **Part I**; solubility evaluation in extreme solution condition: Seven samples (n=7) from each group were introduced in containers containing a freshly prepared buffered Citric acid solution of pH=3 at 37℃, with a sample liquid ratio of 1g/20ml to simulate worst-case exposure to tissue environment. The containers were placed in a shaking incubator (Biotech tech company for medical & laboratory equipment, Cairo, Egypt) for 5 days.[26,27] **Part II**; solubility evaluation in simulation solution condition: Seven samples (n=7) from each group were introduced in containers containing a freshly prepared TRIS-HCL buffer solution of pH value of 7.4 at 37℃, with a sample liquid ratio of 1g/20ml to simulate a more normal body condition. The containers were placed in a shaking incubator at 37℃ for 1-,3- & 5 days.[26,27]

For both tests: pH values were measured at 0h & at the end of each period. Then the average values were reported & analyzed.[28]

The filtrates at each time point were filtered and then centrifuged (Soiiw Electric Lab Benchtop Centrifuge Machine, China) for 30 minutes at 4000 rpm, to separate any residual material. Then the filtrates of centrifuged samples, blank Tris-HCL buffer solution & blank buffered Citric acid solution were analyzed for Ca^{2+} concentration using Atomic Absorption Spectrophotometer (AAS) (SavantAA, GMC,1/2 Naxos Way Keysborough Victoria, 3173 Australia). The concentrations versus the time curve were determined.

The conversion between (ppm) & mass of the element released (mg) was based on the equation:[26,27]

Ca^{2+}conc. in filtrate(ppm)-Ca^{2+}conc. in blank vehicle(ppm)x Immersion solution volume(ml)= Mass of Ca^{2+}content(mg released from 1g specimen in 20ml solution at 37℃

Readings were recorded three times to avoid any discrepancy.

pH test

Tests were performed following ISO 13175-3:2012, part 3,[28] with slight modification. pH was assessed during solubility testing via the same apparatus used for the simulation test. pH was measured at 0h, 24h, 72h & 120h of immersion with a previously calibrated pH meter (Orion™ Versa Star Pro™ Benchtop pH Meter, Thermo Scientific, USA.) To avoid discrepancy each measurement was recorded 3 times. The mean pH value at each time & their changes with immersion time were reported & analyzed.

Statistical Analyses

The mean & standard deviation values were calculated for all groups and tests. Data were explored for normality using Kolmogorov-Smirnov & Shapiro-Wilk tests. One-way ANOVA followed by Tukey post hoc test were used to compare between more than two groups in non-related samples. Repeated measure ANOVA was used to compare between more than two groups in related samples. A paired sample t-test was used to compare between two groups in related samples. The significance level was set at $p \leq 0.05$.

Statistical analysis was performed using IBM® SPSS® Statistics Version 20 for Windows.

Results

Radiopacity Results

The greatest mean grey values were recorded in group I (117.456) & HD group (113.046) which were equivalent to one aluminum step-wedge, then the least values were recorded by BC group (104.329), group II (99.9718) & group III (99.5961) that were equivalent to less than number one aluminum step-wedge. A statistically significant difference was recorded amongst all groups(p<0.001) and in pairwise comparison between group I and all other groups except for the HD group (p<0.001). Also, in pairwise comparison between group II & HD (p=0.004) and group III & HD group (p=0.003). Otherwise, no statistically significant differences were recorded. (Figure 2)

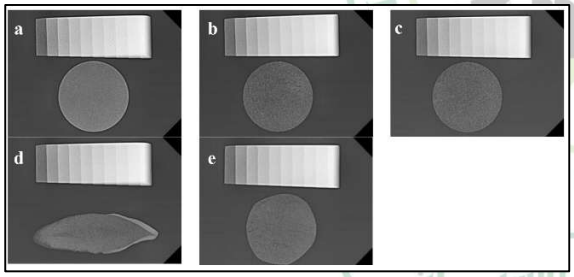

Figure 2: Radiographic images of studied groups along with an aluminum step wedge: a. group I, b. group II, c. group III, d. HD group., e. BC group.

Solubility Results

The concentration & calculated mass of Ca^{2+} released from different groups in Tris-HCL buffer solution for 1-, 3- & 5 days & Citric acid buffer solution for 5 days were collected & tabulated. (Tables 1,2)

Effect of time on different groups

i) Extreme Solution/Citric Acid

Detection of a statistically significant increase in Ca^{2+} concentration & mass between groups I, II, & III when placed in extreme solution, & when placed in the simulation solution at 5-days timepoint, (p<0.001)

Table 1: Means & standard deviation (SD) values of Ca^{2+} concentrations (ppm) in the TRIS-HCl and buffered Citric acid solutions wherein different groups were immersed.

Groups	*Tris-HCL*			*p-value*	*Citric acid*
	Day 1	**Day 3**	**Day 5**		**Day 5**
nHA	8.40 cC ± 0.41	32.98 bC ± 3	129.17 aC ± 19.69	<0.001*	1594.29 C ± 44.29
2 nHA:1ω3	160.00 cB ±17.52	228.50 bB ±72.02	638.21 aB ± 16.51	<0.001*	2070.71 B ± 48.32
1 nHA:1ω3	358.43 cA ±19.69	465.64 bA ± 35.6	794.79 aA ± 18.15	<0.001*	2336.36 A ± 58.29
p-value	**<0.001***	**<0.001***	**<0.001***		**<0.001***

Means with different small letters in the same row indicate significant differences and means with different capital letters in the same column indicate significant differences. *; significant (p<0.05)

Table 2: Means & standard deviation (SD) values of Ca^{2+} mg/kg released in the TRIS-HCl and buffered Citric acid solutions wherein different groups were immersed.

Groups	Tris-HCL			p-value	Citric acid
	Day 1	**Day 3**	**Day 5**		**Day 5**
nHA	0.23 ^{bC} ± 0.12	0.60 ^{bC} ± 0.02	2.52 ^{aC} ± 0.15	**<0.001***	31.85^{C} ± 0.33
2 nHA:1ω3	3.14 ^{cB} ± 0.13	4.51 ^{bB} ± 0.54	12.70 ^{aB} ± 0.12	**<0.001***	41.38 ^{B} ± 0.37
1 nHA:1ω3	7.11 ^{cA} ± 0.15	9.25 ^{bA} ± 0.27	15.76 ^{aA} ± 0.15	**<0.001***	46.69 ^{A} ± 0.44
p-value	**<0.001***	**<0.001***	**<0.001***		**<0.001***

Means with different small letters in the same row indicate significant differences and means with different capital letters in the same column indicate significant differences. *; significant (p<0.05)

ii) Simulation Solution/Tris-HCL

In the simulation solution test, the results showed that the greatest mean of Ca^{2+} concentration was recorded at 5-days timepoint followed by 3-days & 1-day timepoints in all three test groups where group I recorded:129.17, 32.98 & 8.40 ppm respectively, group II recorded: 638.21, 228.50 & 160.00ppm respectively, & group III recorded: 794.79, 465.64 & 358.43ppm respectively. A statistically significant difference between the three timepoints readings (p<0.001) was recorded within each group. Also, a statistically significant difference was found when pairwise comparisons were performed between all timepoints readings. While the corresponding calculated masses of Ca^{2+} at 1, 3 & 5 days of immersion were 0.23, 0.60, and 2.52 mg

respectively in group I, 3.14, 4.51, and 12.70 mg respectively in group II & 7.11, 9.25, 15.76 mg respectively in group III with a statistically significant difference amongst all three timepoints (p<0.001) within each test group. Except for the pairwise comparison between 3-days timepoint readings & 1-day timepoint readings within group I, other statistically significant differences were found upon pairwise comparing between all timepoints readings within each test group (p<0.001).

Relation between groups at different timepoints

i) Extreme Solution/Citric acid

In this scenario, the readings were recorded at only one timepoint (5-days timepoint), at this time point the greatest mean of Ca^{2+} concentration and mass were recorded in group III, followed by group II, & then group I with statistically significant difference amongst the three test groups (p<0.001).

ii) Simulation Solution/Tris-HCL

At all timepoints the results showed that the greatest mean of Ca^{2+} concentration was recorded in group III (1nHA:1ω_3) followed by group II (2nHA:1ω_3) & group I (nHA) showing evidence of a statistically significant difference amongst the three test groups (p<0.001). At the 1-day timepoint the readings were respectively: 358.43, 160.00 & 8.40ppm. At the 3-day timepoint the readings, respectively, were: 465.64, 228.50 & 32.98 ppm. While at the 5-days timepoint the readings were respectively: 794.79, 638.21 & 129.17 ppm. Also, a statistically significant difference was found when pairwise comparisons were performed between all test groups at each timepoint (p<0.001).

At all timepoints, the results showed that the greatest mean of Ca^{2+} masses was recorded in group III followed by group II & group I, showing evidence of a statistically significant difference amongst the three test groups (p<0.001). At the 1-day timepoint the readings were respectively: 7.11, 3.14, 0.23mg. At the 3-day timepoint, the readings, respectively, were: 9.25, 4.51, and 0.60 mg. While at the 5-day timepoint, the readings were respectively: 15.76, 12.70, and 2.52 mg. Also, a statistically significant difference was found when pairwise comparisons were performed between all test groups at each timepoint (p<0.001).

pH value Results

In group I, there was an initial increase in the pH at 0h (7.94) followed by a gradual decrease over time (7.75 at 1 day, 7.61 at 3 days & 6.72 at 5 days). Unlike group I, groups II & III showed an opposite behaviour of an initial decrease in pH at 0h (group II: 5.04 & group III:4.67) followed by a gradual increase over time (group II: 5.38 & group III: 4.88 at 1-day, group II: 5.87 & group III: 5.16 at 3-days & group II: 6.07 & group III: 5.33 at 5-days). A statistically significant difference was recorded amongst all timepoints readings within each test group (p<0.001). (Figure 3)

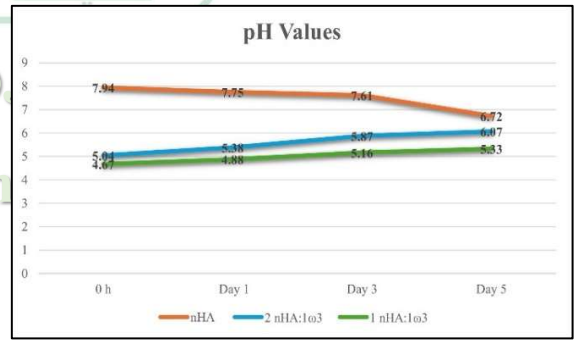

Figure 3: pH values wherein different groups are immersed.

Discussion

A certain level of radiopacity is required in bone graft materials, the density of the material should be distinguished in radiographic evaluation with clear distinction from the surrounding tissues during the healing process.[24,29]

The current study showed that the highest radiopacity levels were recorded in the Nano-Hydroxyapatite group which was higher than human dentin (but with no statistically significant difference), & significantly higher than bovine cortical bone. While groups containing Omega-3 showed a decreased mean grey value than the Nano-Hydroxyapatite group, human dentin, & cortical bone but with no statistically significant difference with the latter. Also, Group II was more radiopaque than Group III but with no statistically significant difference. This may be attributed to the increased content of Nano-Hydroxyapatite in Group II than in Group III.

The radiopacity of Nano-Hydroxyapatite was also reported by other studies where they used it as a bioactive radiopacifier imparting radiopacity to platelet-rich plasma.[30]

Other studies disagreed and reported that nHA required a radiopacifier to be more radiographically distinguishable.[22] Also, it was less radiopaque than cortical bone & had higher radiopacity than the human dentin sample.[24] It is worth mentioning that the difference in results might be due to the difference in methodology used to prepare the samples. In our study, pellets of Groups I, II & III were prepared by pressing the powder in a manual pellet pressing machine under 5tons of 1mm in thickness to achieve a denser form of light nanomaterial, therefore; more radiopaque. Also, the bovine cortical bone sample & the human dentin sample were cut into 1mm thickness, while in Pekkan et al's[24] study, the bovine cortical bone sample was cut into 3 mm samples & all bone graft samples were not pressed & were prepared by filling in a wax mould of 3mm in thickness. Only the human dentin sample was cut to a thickness of 1mm.

As Omega-3-containing groups were less radiopaque than cortical bone, it is recommended that it would not be used in defects surrounded by cortical bone. As the cortical bone may hamper the radiographic appearance of the graft & follow-up will be problematic.[24] Therefore, future studies dealing with imparting radiopacity to Omega-3-containing groups, are recommended.

Another important property of bone graft substitutes is solubility. Solubility testing through the analysis of the Calcium ion mass released was performed in the current study, following the approach of Yang et al[27] (2020). They evaluated the solubility of a new synthetic bone substitute material by calculating the mass of calcium ions degraded in buffered Citric Acid solution simulating a bacterial infection condition where pH is low & Tris-HCL solution simulating body fluid.

Upon evaluation of solubility in the current study, results showed that within the Tris-HCL group, the solubility of calcium ions increased with increased immersion time in all groups. A study by Ramesh & Sadasivan[31] (2017) also reported the same result.

Also, the current results of this study showed that the solubility of calcium ions increased with the decrease in the pH of the solution at the same time interval of 5 days. This was in agreement with Hankermeyer et al[32] (2002), who stated that the dissolution of carbonated hydroxyapatite increased with the decrease in pH of the solution (increase in H^+). Similarly, other studies stated that when the pH increased from 4.0 to 7.0, the concentrations of calcium released from Nano-Hydroxyapatite significantly decreased.[33,34]

At all observation time points, & in both simulation & extreme solutions, Group III (1nHA:1ω3) showed the greatest mean of calcium concentration released compared to Group II (2nHA:1ω3) & Group I (nHA). This might be attributed to the increased content of Omega-3 in this group, which caused more decrease in the pH of solutions leading to more dissociation of calcium ions.

Dissolution products of any implanted graft can affect the pH of the local microenvironment, affecting cell function, metabolism, bone formation & mineralization.[35] Therefore, in the current study, the change in pH of the solution in which the graft was placed was tested using a pre-calibrated pH meter.

Regarding the change in pH of the dissolution medium, the current study's results showed that Nano-Hydroxyapatite caused an initial rise in pH up to 7.94 when placed in Tris HCL solution of pH 7.4 at 0h. Then, the pH of the solution gradually dropped from day 1 to day 5 reaching a pH value of 6.72 which is considered close to the physiological pH. This was in agreement with Bovand et al[36] (2019), who stated that the pH of Simulated Body Fluid (SBF) solution increased up to 7.8 at early immersion time in groups containing 30% Hydroxyapatite, then, at a later stage, longer immersion time caused the pH of the composition to decrease gradually. Also, in 2021, Thakare et al[37] found that the degradation of Hydroxyapatite pellet samples in Tris-HCL solution at pH 7.4 & temperature 37°C, caused the pH of the buffer solution to increase from 7.4 to 7.8, which confirms the biodegradation of Hydroxyapatite. But contradictory to the current study; the rise in pH continued for four weeks.

Group II & Group III showed comparable pH results when immersed in Tris-HCL, where they both had an initial decrease in pH value up to 5.04 for Group II & 4.67 for Group III. Then, the pH value gradually increased from day 1 to day 5 reaching a pH value of 6.07 for Group II & 5.33 for Group III. This could be explained by the effect of adding Omega-3 - a fatty acid- containing an acidic hydrogen because of their carboxylic acid (COOH) functional groups. Thus, the gradual increase in the pH might be attributed to the dissociation of Nano-Hydroxyapatite & the release of Hydroxide ions.[38]

Similarly, a study by Nedeljkovic et al[39] in 2016, showed that Hydroxyapatite increased the start pH value of all solutions of distilled water with different pH values adjusted to approximately 4, 5, 6, & 7. Also, the same result was obtained in supernatant containing S. mutans–produced acids. Likewise, Cieplik et al[40] (2020) observed the release of Ca^{2+} ions from synthetic Hydroxyapatite upon bacterial acid challenge & showed some buffering ability.

In the current in-vitro study, one limitation is that the solubility test was conducted in a thermodynamically closed system. While, in the in vivo condition, grafts are placed in an open system where there is an interchangeable flow of microenvironment solutions around the graft material. Therefore, the results of this test should be interpreted with care. Another limitation of this study is the short observation time. A longer time of observation is recommended in further studies.

Conclusion

1- Adding omega 3 powder to nanohydroxyapatite lowered its radiopacity & increased its solubility in tris-HCL of 7.4 pH and did not affect its calcium release.

2- Nano-hydroxyapatite showed increased solubility in an acidic medium. This may have a clinical significance, that nHA performance is not affected by the presence of acidic bacterial products. In fact, it caused a buffering effect to their acidic medium.

References

1. Kubasiewicz-Ross P, Hadzik J, Seeliger J, Kozak K, Jurczyszyn K, Gerber H, et al. New nano-hydroxyapatite in bone defect regeneration: A histological study in rats. Ann Anat. 2017;213:83-90. Doi: 10.1016/j.aanat.2017.05.010.

2. Ku JK, Kim YK, Yun PY. Influence of biodegradable polymer membrane on new bone formation and biodegradation of biphasic bone substitutes: an animal mandibular defect model study.

Maxillofac Plast Reconstr Surg. 2020;42(1):34. Doi: 10.1186/s40902-020-00280-5.
3. Radha G, Manjubaashini N, Balakumar S. Nano-hydroxyapatite/natural polymer composite scaffolds for bone tissue engineering: a brief review of recent trend. In vitro models. 2023;2:125–151. https://doi.org/10.1007/s44164-023-00049-w.
4. Wei S, Ma JX, Xu L, Gu XS, Ma XL. Biodegradable materials for bone defect repair. Military Medical Research. 2020;7(1):54. Doi: 10.1186/s40779-020-00280-6.
5. Salmasi S, Nayyer L, Seifalian AM, Blunn GW. Nanohydroxyapatite Effect on the Degradation, Osteoconduction and Mechanical Properties of Polymeric Bone Tissue Engineered Scaffolds. Open Orthop. J. 2016;10:900-19. Doi: 10.2174/1874325001610010900.
6. Pushpalatha C, Gayathri VS, Sowmya S V., Augustine D, Alamoudi A, Zidane B, et al. Nanohydroxyapatite in dentistry: A comprehensive review. Saudi Dent J. 2023;35(6):741-52. Doi: 10.1016/j.sdentj.2023.05.018.
7. Lock J, Nguyen TY, Liu H. Nanophase hydroxyapatite and poly(lactide-co-glycolide) composites promote human mesenchymal stem cell adhesion and osteogenic differentiation in vitro. J. Mater. Sci. Mater. Med. 2012;23(10):2543-52. Doi: 10.1007/s10856-012-4709-0.
8. Webster TJ, Ergun C, Doremus RH, Siegel RW, Bizios R. Specific proteins mediate enhanced osteoblast adhesion on nanophase ceramics. J Biomed Mater Res. 2000;51(3):475-83. Doi:10.1002/10974636(20000905)51:3<475:aidjbm23>3.0.co;2-9.
9. Cai Y, Liu Y, Yan W, Hu Q, Tao J, Zhang M, et al. Role of hydroxyapatite nanoparticle size in bone cell proliferation. J Mater Chem. 2007;17(36):3780-87. Doi:10.1039/b705129h.
10. Pezzatini S, Morbidelli L, Solito R, Paccagnini E, Boanini E, Bigi A, et al. Nanostructured HA crystals up-regulate FGF-2 expression and activity in microvascular endothelium promoting angiogenesis. Bone. 2007;41(4):523-34. Doi: 10.1016/j.bone.2007.06.016.
11. Brandt J, Henning S, Michler G, Hein W, Bernstein A, Schulz M. Nanocrystalline hydroxyapatite for bone repair: An animal study. J Mater Sci Mater Med. 2010;21(1):283-94. Doi: 10.1007/s10856-009-3859-1.
12. Kajarabille N, Díaz-Castro J, Hijano S, López-Frías M, López-Aliaga I, Ochoa JJ. A new insight to bone turnover: Role of ω -3 polyunsaturated fatty acids. Scientific World Journal. 2013;2013(2):589641. Doi: 10.1155/2013/589641.
13. Bao M, Zhang K, Wei Y, Hua W, Gao Y, Li X, et al. Therapeutic potentials and modulatory mechanisms of fatty acids in bone. Cell Prolif. 2020;53(2):e12735. Doi: 10.1111/cpr.12735.
14. Kruger MC, Coetzee M, Haag M, Weiler H. Long-chain polyunsaturated fatty acids: Selected mechanisms of action on bone. Prog Lipid Res. 2010;49(4):438-49. Doi: 10.1016/j.plipres.2010.06.002.
15. Calder PC. Mechanisms of action of (n-3) fatty acids. J Nutr. 2012;142(3):592S-99S. Doi: 10.3945/jn.111.155259.
16. Raizada MK, Sable DM, Chowdhery A, Chavan MS, Rajpurohit LS. Omega 3: a novel treatment agent in oral submucous fibrosis: a pilot study. J Oral Pathol Med. 2017;46(6):439-42. Doi: 10.1111/jop.12542.
17. Arias Z, Nizami MZI, Chen X, Chai X, Xu B, Kuang C, et al. Recent Advances in Apical Periodontitis Treatment: A Narrative Review. Bioengineering. 2023;10(4):488. Doi: 10.3390/bioengineering10040488.
18. Ferreira I, Falcato F, Bandarra N, Rauter AP. Resolvins, Protectins, and Maresins: DHA-Derived Specialized Pro-Resolving Mediators, Biosynthetic Pathways, Synthetic Approaches, and Their Role in Inflammation. Molecules. 2022;27(5):1677. Doi: 10.3390/molecules27051677.
19. Albuquerque-Souza E, Schulte F, Chen T, Hardt M, Hasturk H, Van Dyke TE, et al. Maresin-1 and Resolvin E1 Promote Regenerative Properties of Periodontal Ligament Stem Cells Under Inflammatory Conditions. Front Immunol. 2020;11:585530. Doi: 10.3389/fimmu.2020.585530.
20. International Organization for Standardization. ISO 13116:2014(en). Dentistry — Test Method for Determining Radio-Opacity of Materials. Geneva:ISO;2014.
21. Mohn D, Zehnder M, Imfeld T, Stark WJ. Radio-opaque nanosized bioactive glass for potential root canal application: evaluation of radiopacity, bioactivity and alkaline capacity. Int Endod J. 2010 Mar;43(3):210-7. doi: 10.1111/j.1365-2591.2009.01660.x.
22. Ajeesh M, Francis BF, Annie J, Varma PRH. Nano iron oxide-hydroxyapatite composite ceramics with enhanced radiopacity. J Mater Sci Mater Med. 2010;21(5):1427-34. Doi: 10.1007/s10856-010-4005-9.
23. Clavijo Mejía GA. Development of Radiopaque Biohydroxyapatite / Bioglass Coatings Deposited by Thermal Spray for Biomedical Applications. Matériaux. Universitéde Limoges; El Centro de Investigacion y de Estudios Avanzados del Insituto Politécnico National. Cinvestav (Méxique);2019. Français. NNT: 2019LIMO0099.
24. Pekkan G, Aktas A, Pekkan K. Comparative radiopacity of bone graft materials. J Craniomaxillofac

Surg. 2012;40(1):e1-4. Doi: 10.1016/j.jcms.2011.01.018.
25. Oliveira L V., da Silva GR, Souza GL, Magalhães TEA, Barbosa GLR, Turrioni AP, et al. A laboratory evaluation of cell viability, radiopacity and tooth discoloration induced by regenerative endodontic materials. Int Endod J. 2020;53(8):1140-52. Doi: 10.1111/iej.13308.
26. International Organization for Standardization. ISO 10993-14. Biological evaluation of medical devices — Part 14: Identification and quantification of degradation products from ceramics. Geneva:ISO;2001.
27. Yang BC, Lee JW, Ju CP, Lin JHC. Physical/chemical properties and resorption behavior of a newly developed Ca/P/S-based bone substitute material. Materials. 2020;13(16):3458. Doi: 10.3390/ma13163458.
28. International Organization for Standardization. ISO 13175-3:2012. Implants for surgery — Calcium phosphates — Part 3: Hydroxyapatite and beta-tricalcium phosphate bone substitutes. Geneva:ISO;2012.
29. No YJ, Nguyen T, Lu Z, Mirkhalaf M, Fei F, Foley M, et al. Development of a bioactive and radiopaque bismuth doped baghdadite ceramic for bone tissue engineering. Bone. 2021;153:116147. Doi: 10.1016/j.bone.2021.116147.
30. Mahendran K, Kottuppallil G, Sekar V. Comparative evaluation of radiopacity and cytotoxicity of platelet-rich fibrin, platelet-rich fibrin + 50wt% nano-hydroxyapatite, platelet-rich fibrin + 50wt% dentin chips: An in vitro study. J Conserv Dent. 2019;22(1):28-33. Doi: 10.4103/JCD.JCD_281_18.
31. Ramesh R and Sadasivan A. Comparative evaluation of the physical and chemical properties of three different alloplastic bone graft materials used in periodontal regeneration. Int J Curr Res. 2017;9(9):57220-22.
32. Hankermeyer CR, Ohashi KL, Delaney DC, Ross J, Constantz BR. Dissolution rates of carbonated hydroxyapatite in hydrochloric acid. Biomaterials. 2002;23(3):743-50. Doi: 10.1016/s0142-9612(01)00179-x.
33. Huang S, Gao S, Cheng L, Yu H. Remineralization potential of nano-hydroxyapatite on initial enamel lesions: An in vitro study. Caries Res. 2011;45(5):460-8. Doi: 10.1159/000331207
34. Miyajima H, Touji H, Iijima K. Hydroxyapatite particles from simulated body fluids with different ph and their effects on mesenchymal stem cells. Nanomaterials. 2021;11(10):2517. Doi: 10.3390/nano11102517.
35. Monfoulet LE, Becquart P, Marchat D, Vandamme K, Bourguignon M, Pacard E, et al. The pH in the microenvironment of human mesenchymal stem cells is a critical factor for optimal osteogenesis in tissue-engineered constructs. Tissue Eng Part A. 2014;20(13–14):1827-40.
36. Bovand D, Allazadeh MR, Rasouli S, Khodadad E, Borhani E. Studying the effect of hydroxyapatite particles in osteoconductivity of Ti-HA bioceramic. J Aust Ceram Soc. 2019;55(6):395-403. DOI:10.1007/s41779-018-0247-7.
37. Thakare VG, Bhatkar VB, Wadegaonkar PA, Omanwar SK. Wet Chemical Synthesis, Characterization and Biocompatibility Study of Hydroxyapatite Used As Biomaterials. Int J Sci Res Sci Technol. 2021;8(1):464–70. Doi: 10.32628/IJSRST.RAMAN2181.
38. Min JH, Kwon HK, Kim BI. The addition of nano-sized hydroxyapatite to a sports drink to inhibit dental erosion - In vitro study using bovine enamel. J Dent. 2011;39(9):629-35. Doi: 10.1016/j.jdent.2011.07.001.
39. Nedeljkovic I, De Munck J, Slomka V, Van Meerbeek B, Teughels W, Van Landuyt KL. Lack of Buffering by Composites Promotes Shift to More Cariogenic Bacteria. J Dent Res. 2016;95(8):875-81. Doi: 10.1177/0022034516647677.
40. Cieplik F, Rupp CM, Hirsch S, Muehler D, Enax J, Meyer F, et al. Ca2+ release and buffering effects of synthetic hydroxyapatite following bacterial acid challenge. BMC Oral Health. 2020;20(1):85. Doi: 10.1186/s12903-020-01080-z.