Effects of incorporation of nano-fluorapatite particles on microhardness, fluoride releasing properties, and biocompatibility of a conventional glass ionomer cement (GIC)

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Present study evaluated effects of addition of Nanoparticles fluorapatite (Nano-FA) on microhardness and fluoride release of a Glass Ionomer Cement (GIC, Fuji IX GP Fast). Forty-eight specimens prepared, divided equally into 4 groups (2 with Nano-FA); after 24 h and one week Vickers microhardness (HV) was measured. Nano-FA specimens were made from addition of nano-FA to Fuji IX powder (glass powder/Nano-FA ratio=20:1 wt/wt, 3.6:1 P/L ratio). At 24 h, mean (95% CI) HV for GIC and Nano-FA GIC were 40.59 (39.51–41.66) and 46.89 (45.95–47.82) kg/mm², and at one week 44.98 (44.23–45.72), 53.29 (52.58–53.99) kg/mm², respectively. Findings indicated higher HV in Nano-FA specimens (F=221.088, p<0.001). Twenty-eight days weekly cumulative fluoride release in both groups was not different (p>0.05). MTT assay exhibited no inhibition of cell proliferation or reduction in metabolic activity in experimental [84.0 (3.3)] or control groups [85.1 (4.7)] with no difference between groups (p>0.05). New nano-FA GIC was biocompatible and showed improved surface hardness. Future clinical trials can verify the usefulness of Nano-FA GIC.

**Keywords**: Glass ionomer cement, Nano-fluoroapatite, Microhardness test, Fluoride release, MTT assay

INTRODUCTION

Glass ionomer cement (GIC) was invented in early 1970's, a water-based cement known as polyalkenoate cement, possessing unique properties such as fluoride release (anti-cariogenic action), adhesion to tooth structure and base metals, and desirable coefficient of thermal expansion, and biocompatibility. However, GICs suffer from low mechanical properties, brittleness, unfavorable appearance, and moisture sensitivity in the early stages of the placement. These disadvantages prevent broader clinical applications of GICs, as they are not as durable as resin composites in everyday dentistry. Although stronger GICs with tooth-like appearance and improved handling characteristics are now available, lack of strength and toughness are still major problems.

Synthesis and characterization of nanoparticles of hydroxyapatite (HA) and fluoroapatite (FA) and incorporation of these nanoparticles in the composition of the conventional GIC (Fuji II GC) has been reported. The addition of synthesized nanoparticles of HA and FA into GIC composition enhanced the mechanical properties (e.g. compressive, diametral tensile and biaxial flexural strength) of GIC. Nanoparticles of fluoroapatite enhanced the mechanical properties more than HA counterparts, a phenomenon that was contributed to the lower solubility rate of FA in water in comparison to HA, when added to GIC. FA nanoparticles appear promising additives for glass ionomer restorative dental materials.

The effects of incorporation of nano-FA on other important clinically relevant properties such as hardness and fluoride releasing properties of GICs have yet to be addressed. Therefore, the objectives of this study were to synthesize FA nanoparticles and to evaluate the effect of incorporation of the nanoparticles on physical properties of GICs such as microhardness and fluoride releasing properties. The tested null hypotheses of the current study were: (I) No difference would be found between the weekly cumulative fluoride release of nano-FA GIC and the control group (Fuji IX); (II) No difference would be found between the microhardness values of nano-FA GIC and the control group; and (III) No difference would be found between the biocompatibility of nano-FA GIC and the control group.

MATERIALS AND METHODS

**Specimen preparation**

The control group was the mixture of the powder and liquid of the commercially available Fuji IX GP Fast (Composition: Alumino-fluoro-silicate glass, polyacrylic acid, distilled water, polybasic carboxylic acid). Nanoparticles of fluorapatite were synthesized...
and characterized using (NH4)2HPO4, Ca (NO3)2•4H2O, and ammonium fluoride (NH4F) (Sigma) via a well-established ethanol-based sol–gel technique9. The glass powder and liquid from a glass ionomer cement (Fuji IX GP Fast, GC America, Alsip, IL, USA) were used. In order to prepare nano-FA-containing glass powders, an appropriate amount (glass powder/FA ratio of 20:1 wt/wt) of glass ionomer powder, and nano-FA were weighed accurately and mixed. The powder/liquid (P/L) ratio of 3.6/1 was used as recommended by the manufacturer. The above mixing ratios were chosen based on previous preliminary experiments. Different ratios were evaluated and the current ratio showed enhanced mechanical properties while the cement was still workable and working/setting time was not adversely affected.

Forty-eight cylindrical specimens (6×3 mm) were prepared using a PTFE (polytetrafluoroethylene) mold at 37°C, and stored in distilled water at 37°C for up to 1 week. Subsequently, equal specimens were fabricated for each group. The molds were filled with the cement mixture and covered with a piece of film and with a glass slide. Hand pressure was applied for 20 s while excess material was extruded from the top of the mold. The specimens were removed from the molds after 20 min and conditioned in distilled water at 37°C for up to 1 week. Commercial Fuji IX was used as the control group for comparison.

The Scanning Electron Microscopy (SEM) of the prepared powder was also performed to check the sizes of the nano-FA particles (Figs. 1a and b).

**Microhardness assessment**

The Vickers hardness (HV) of the experimental GIC specimens and the control group (commercially available GIC) was determined using a microhardness tester (Model MVK-E, M 400, LECO, St. Joseph, MI, USA). A diamond indenter with 100 g load and a dwell time of 10 s were utilized. The Vickers hardness values for each specimen was calculated according to the following equation10:

\[
HV = \frac{2L \times \sin(\theta/2)}{d^2}
\]

Where, \(L\) = applied load (kg), \(\theta\) = angle of 136°, and \(d\) = mean diagonal length (mm). Since \(\sin(\theta/2) = 1.8544\). The Vickers hardness value (HV) has units of kg/mm².

**Weekly cumulative fluoride release**

Fluoride releasing properties of nano-FA GIC was measured, over 28 days, using Ion (Fluoride)-Selective Electrode (ISE) model Thermo-Orion Ionplus according to previously published methods11,12. Prior starting the measurements, electrode piece was rinsed with distilled water and filled with electrode filling solution (Orion Ionplus®, Thermo Orion, Thermoscientific, Carlsbad, CA, USA). The ISE was calibrated by using standard fluoride preparations with deionized water. Standards solutions for de-ionized water were made by serial dilution of 0.1 mM aqueous sodium fluoride solution (Orion Sodium Fluoride Standard) to give concentrations of 0.001, 0.01, 0.1, 1.0 and 10 mM solutions. The meter/electrode combination was calibrated using a range of standard solutions from 0.001 to 10.0 mg/L. Both standards and specimen solutions were treated in the same way, 0.4 mL were removed from the storage water and mixed with 2.00 mL TISAB IV buffer (Total Ionic Strength Adjustment Buffer) and 1.65 mL de-ionized water. A reading was taken after 2 min of continuous stirring.

The 24 h and weekly cumulative fluoride release was calculated for each specimen by adding the amount of fluoride released from the consecutive elution for up to one week. A cumulative release (µg•cm⁻²) curve against time was then plotted for each group.

**C2C12 culture and MTT assay**

In order to analyze the cytotoxicity of our experimental glass-ionomer, mouse myoblast cell line C2C12
Table 1 Two-way ANOVA with Bonferroni post-hoc test for comparison of the Vickers microhardness (HV) scores (kg/mm²) in different groups

| Groups                  | Mean Difference, HV (kg/mm²) | p    | 95% Confidence Interval |
|-------------------------|-------------------------------|------|-------------------------|
| GIC*, 24 h              | GIC, 1 week                   | −6.30| 0.000                   | −7.7907 − 4.8093 |
| GIC, 24 h               | Nano-FA GIC, 24 h             | −4.39| 0.000                   | −5.8327 − 2.9459 |
| GIC, 24 h               | Nano-FA GIC, 1 week           | −12.70| 0.000                  | −14.1452 − 11.2584 |
| GIC, 1 week             | Nano-FA GIC, 24 h             | 1.91 | 0.005                   | 0.4673 3.3541    |
| GIC, 1 week             | Nano-FA GIC, 1 week           | −6.40| 0.000                   | −7.8452 − 4.9584 |
| Nano-FA GIC, 24 h       | Nano-FA GIC, 1 week           | −8.31| 0.000                   | −9.7069 − 6.9181 |

* Fuji IX GP Fast, GC America, Alsip, IL, USA
Deionized water in comparison to the control group (Fig. 2). Therefore, the null hypothesis for this part of the study was not rejected.

**Biocompatibility analysis**

Our MTT assay exhibited no inhibition of cell proliferation [mean(SD)] or reduction in metabolic activity in the experimental [84.0(3.3)] or control group [85.1(4.7)]. No significant difference in cell proliferation was observed between the two tested groups ($p>0.05$) (Fig. 3).

**DISCUSSION**

Our obtained data confirmed that the first null hypotheses was fully rejected. A statistically significant difference was observed between the microhardness values of nano-FA GIC and the control group (Fuji IX). A significant increase was not observed in the weekly cumulative fluoride releasing of the experimental GIC specimens in comparison to the control group and second null hypothesis was not rejected. Similarly, the third null hypothesis was accepted since our MTT assay failed to show any significant difference between the two tested groups ($p>0.05$) (Fig. 3).

GICs provide sustained fluoride release, which can prevent secondary and recurrent caries. Fluoride ions release occurs via fluoride ion diffusion from the bulk of the OH group radius (1.4 Å); the OH groups of hydroxyapatite crystals of enamel can be replaced by fluoride ions to form fluorapatite. Due to lower solubility and lower crystal energy of fluorapatite in comparison to hydroxyapatite, enamel which uptakes fluoride at hydroxyl sites is more caries resistant and more durable against bacterial acid attacks. Fluoride enhances the crystallinity of the hydroxyapatite crystals and affects the composition of bacterial plaque, which may alter carbohydrate metabolism in it. The mechanism of fluoride release is dominated by diffusion mechanism, where the rate of fluoride release is reduced as time passes according to concentration gradient. Fluoride release involves a two step procedure: a rapid surface elution followed by slower continuous bulk diffusion of fluoride ions. The application of acid conditioner and dentin primer also increased the depth of fluoride uptake in GIC restorations.

We measured the weekly cumulative fluoride release, and the addition of nano-FA particles to the composition of conventional GIC increased the amount of fluoride release from GIC. However, this increase in was not significantly different from the values observed for the control group.

The nano-FA GIC specimens showed significantly greater Vickers microhardness values compared with Fuji IX control group. Moreover, a significant increase in the microhardness of the nano-FA specimens was found after storage in distilled water for a week. The possible explanation is the extraction of low concentration of calcium ions from FA nanoparticles after mixing the powder with a GIC liquid, leading to higher degrees of acid-base reaction within the structure of the setting cement. As a result more polysalt bridges will be formed to the high concentration of phosphate and calcium ions, which reinforce the GIC matrix. Due to the presence of higher number of available hydroxyl, phosphate and fluoride ions in the matrix, the possibility of the formation of hydrogen bonds may be greater, leading to improved interaction between the organic and inorganic networks of the final set cement. As the cement ages in distilled water, there might be more cross-linking, leading to increased Vickers microhardness values. The nanoparticles of FA can occupy the empty spaces between the glass ionomer glass particles, which can further reinforce the material the GIC matrix.

MTT assay has long been regarded as the gold standard of cytotoxicity assays and is a colorimetric assay, measuring the reduction of the yellow tetrazolium MTT (3-(4,5-dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide) by mitochondrial succinate Dehydrogenase in metabolically active cells to purple formazan in the mitochondria. The resulting intracellular purple formazan can be solubilized and quantified by spectrophotometric means. The absorbance of the colored solution can be measured at a certain wavelength (usually between 500 and 600 nm) by a spectrophotometer. Although the findings of the cytotoxicity testing of dental materials depended on cell type as well as the assay system used in the study present observation may suggest that the produced nano-FA GIC is not more cytotoxic comparing to conventional GIC tested. However, the neutral red uptake, resazurin reduction and sulforhodamine B assays have been sugesssted as more reliable methods to MTT assay as reflected by the reproducibility and variability of findings among them.

GICs suffer from weak mechanical properties such as brittleness, low strength and toughness and varies method suggested and used to address these drawbacks.
Reinforcement phases such as zirconia, hydroxyapatite, N-vinyl pyrrolidone, N-vinyl caprolactam, fluoroapatite, and HA/ZAO₂ have been used to enhance GIC mechanical properties. GICs are susceptible to water or saliva contamination in the first 10 min of the setting, and to dehydration in the long-term hardening process. Thermo-light-polymerization during setting of GIC has been shown to improve microhardness of GIC, improve marginal adaptation of material on the cavity walls, increase bond strength to enamel, and decrease porosity inside material.

Limitations of the study are that only one weight ratio was tested for nanoparticles of fluorroapatite, and the study period was of relatively short. In future investigations, dimensional stability, water absorption from this type of modified glass ionomer cement should be studied, preferably in prospective clinical trials.

CONCLUSION

Within the limitations of the present study, it could be concluded that conventional glass ionomer dental cement modified by addition of nanoparticles of fluorroaptate showed enhanced surface hardness. The finding should be validated with future clinical trials.

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CONFLICT OF INTEREST

Authors have no conflict of interest to disclose.

REFERENCES

1) Wilson AD, Kent BE. The glass-ionomer cement, a new translucent cement for dentistry. J Appl Chem Biotechnol 1971; 21: 313.
2) Rajabzadeh G, Salehi S, Nemati A, Tavakoli R, Solati Hashjin M. Enhancing glass ionomer cement features by using the HA/YSZ nanocomposite: a feed forward neural network modelling. J Mech Behav Biomed Mater 2014; 29: 317-27.
3) Moshaverinia A, Roopour N, Rehman IU. Synthesis and characterization of a novel N-vinylcaprolactam (NVC) containing acrylic acid terpolymer for applications in glass-ionomer dental cements (GIC). Acta Biomater 2009; 5: 2101-2108.
4) Moshaverinia A, Roopour N, Rehman IU. Synthesis and characterization of novel fast set proline derivative containing glass-ionomer cement with enhanced mechanical properties. Acta Biomater 2009; 5: 498-507.
5) Xie D, Park JG, Faddah M, Zhao J, Khanijoun HK. Novel amino acid-constructed polyalkenoates for dental glass-ionomer restoratives. J Biomater Appl 2006; 2: 147-165.
6) Kao EC, Culbertson BM, Xie D. Preparation of glass ionomer cement using N-acryloyl-substituted amino acid monomers evaluation of physical properties. Dent Mater 1996; 12: 44-51.
7) Yamazaki T, Brantley WA, Culbertson BM, Seghi R, Schricker S. The measure of wear in N-vinyl pyrrolidinone (NVP) glass-ionomer cements. Polym Adv Technol 2005; 16: 113-116.
8) Sidhu S. Glass-ionomer cement restorative materials: a sticky subject? Aust Dent J 2011; 56: 23-30.
9) Moshaverinia A, Ansari S, Moshaverinia M, Roopour N, Darr JA, Rehman IU. Effects of incorporation of hydroxyapatite and fluoroapatite nanobioceramics into conventional glass ionomer cements (GIC). Acta Biomater 2008; 4: 432-440.
10) Moshaverinia A, Ansari S, Movassaghi Z, Billington RW, Darr JA, Rehman IU. The mechanical properties of conventional glass ionomer cements modified with N-vinylpyrrolidone containing polyacids, nano-hydroxyapatite and fluoroapatite. Dent Mater 2008; 24: 1381-1390.
11) Moshaverinia A, Chee WW, Brantley WA, Schricker SR. Surface properties and bond strength measurements of N-vinylcaprolactam (NVC)-containing glass-ionomer cements. J Prostheth Dent 2011; 105: 185-193.
12) Moshaverinia A, Ansari S, Roopour N, Schricker SR, Chee WW. Effects of N-vinylcaprolactam containing polyelectrolytes on hardness, fluoride release and water sorption of conventional glass ionomers. J Prostheth Dent 2011; 105: 323-331.
13) Van Tonder A, Joubert AM, Cromarty AD. Limitations of the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) assay when compared to three commonly used cell enumeration assays. BMC Res Notes 2015; 8: 47. doi:10.1186/s13104-015-1000-8.
14) Moshaverinia A, Roopour N, Billington RW, Darr JA, Rehman IU. Synthesis of N-vinylpyrrolidone modified acrylic acid copolymer in supercritical fluids and its application in dental glass-ionomer cements. J Mater Sci Mater Med 2008; 19: 2705-2711.
15) Xie D, Culbertson BM, Wang G. Microhardness of N-vinylpyrrolidone modified glass-ionomer cements. J Macromol Sci Part A 1998 4: 547-561.
16) Luo J, Billington RW, Pearson GJ. Kinetics of fluoride release from glass components of glass ionomers. J Dent 2009; 37: 495-501.
17) Swartz ML, Phillips RW, Clark HE. Long-term F release from glass ionomer cements. J Dent Res 1984; 63: 158-160.
18) Guida A, Hill RG, Towler MR, Eramo S. Fluoride release from model glass ionomer cements. J Mater Sci Mater Med 2002; 13: 645-649.
19) Moreno EC, Kresak M, Zahradnik RT. Physicochemical aspects of fluoride-apatite systems relevant to the study of dental caries. Caries Res 1977; 11 Suppl 1: 142-171.
20) Tay WM, Braden M. Fluoride ion diffusion from polyalkenoate (glass ionomer) cements. Biomaterials 1988; 9: 454-456.
21) Wang G, Culbertson BM, Xie D, Seghi RR. Physical property evaluations of perfluorotriethylene glycol dimethacrylate as a potential reactive diluent in dental composite resins. J Macromol Sci Pure Appl Chem 1999; 36: 225-236.
22) Tam LE, Chan GP, Yim D. In vitro caries inhibition effects by conventional and resin-modified glass-ionomer restorations. Oper Dent 1997; 22: 4-14.
23) Xie D, Brantley WA, Culbertson BM, Wang G. Mechanical properties and microstructures of glass-ionomer cements. Dent Mater 2006; 16: 129-138.
24) Yap AUJ, Pek YS, Kumar RA, Cheang P, Khor KA. Experimental studies on a new bioactive material: HA ionomer cements. Biomaterials 2002; 23: 955-962.
25) Gavic L, Gorseta K, Borzabadi-Farahani A, Tadin A, Glavina D, van Duinen RN, Lynch E. The effect of thermo-light-curing on the micro-hardness of glass ionomer cements. Int J Periodontics Restorative Dent 2016; 36: 425-430.