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Biological, thermal and mechanical characterization of modified glass ionomer cements: The role of nanohydroxyapatite, ciprofloxacin and zinc L-carnosine

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ABSTRACT

The study evaluated the effects of 4 wt% nanohydroxyapatite (HA), 6 wt% zinc L-carnosine (MDA) and 1.5 wt% Ciprofloxacin (AB) on the mechanical, thermal and biological properties of glass ionomer cements (GIC). Filler and additive concentrations were selected after a previous study had tested single components and different percentages. Specimens included five silicon molds of each GIC cement for all tests. They were stored at room temperature for 24 h from specimen collection to analysis. Mechanical tests, calorimetric analysis, morphological investigation, antibacterial and cell viability assays were conducted. One-way analysis of variance (ANOVA) was used for data analysis with significance set at $p < 0.05$. Adding HA, MDA and AB to GICs modified their thermal, mechanical and microbiological properties. Polymerization increased. A slight decrease in the compressive strength of modified GICs was observed in dry condition ($p < 0.05$). Cement extracts affected cell viability in relation to extract dilution. Mechanical behavior improved in modified glass ionomer cements, especially with the powder formulated antibiotic. Overall cytotoxicity was reduced. Therefore adding nanohydroxyapatite, antibiotic and a mucosal defensive agent to conventional glass ionomer cement in special need patients could improve the clinical, preventive and therapeutic performance of the cements, without altering their mechanical properties.

1. Introduction

In daily clinical practice standard dental filling techniques and materials are often unsuitable for children with special needs or patients who are afraid of the dentist [1]. For these poorly compliant individuals, minimally-invasive approaches to caries removal include lasers [2], ultrasound [3], chemo–mechanical products [4] or sharp excavators [5]. Unfortunately, the risk of failure of these atraumatic restorative techniques (ART), particularly when evaluating multiple restored surfaces, is higher than with standard dental procedures (OR 1.11, 95% CI 0.54 to 2.29) [5] and the survival rates were 62% (CI, 51–73%) and 80% (CI, 76–83%) of, respectively primary and permanent teeth [6].

In ART the most commonly used filling materials are glass ionomer cements (GICs) which are successful in a badly isolated, moist environment, release fluoride to prevent cavity progression and possess adequate physical properties for chewing. First developed in 1970, GICs are the product of an acid-base reaction, whose materials usually consist of an acid degradable fluoro-aluminosilicate glass powder and a carboxylic acid based liquid ionomer. Calcium ions are released from the acid etched glass to form insoluble polysalts [7].

One of the most popular specific fillers that were added to GICs to improve their mechanical and antibacterial features is chlorhexidine [8–14]. Endowed with a slow release antibacterial action, chlorhexidine was associated with less bacterial growth than fluoride. However, at high dosages it inhibited protein synthesis and mitochondrial activity in pulp and gingival cells [12,13].

Hydroxyapatite (HA), a major inorganic part of tooth enamel (97%)

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Footnotes

GIC glass ionomer cement
GIC-HA-MDA-ABp glass ionomer cement-nanohydroxyapatite-mucosal defensive agent-antibiotic powder
GIC-HA-MDA-ABe glass ionomer cement nanohydroxyapatite-mucosal defensive agent-antibiotic emulsion
ART atraumatic restorative treatments

and dentine (70%), is an other popular additive that was reported to improve bond strength to dentine, cement mechanical properties and GIC biocompatibility [15–25]. Its chemical formula is $Ca_{10}(PO_4)_6OH_2$ with a 1.67 calcium to phosphorus ratio (Ca/P).

Finally, a mucosal defense agent (MDA) is another useful additive which might prevent and repair mucous membrane or aphthosis lesions [26–29]. Many MDA were tested e.g. vitamin E and allopurinol [27], sucralfate benzydamine prostaglandin E1 and palifermin [28].

In an attempt to generate a cement with a better mechanical and clinical performance a previous study tested an antibiotic, HA and MDA separately, to establish the optimal concentration of each which resulted as 1.5%AB, 4%HA, and 6% MDA [29].

The present study advanced research into providing better cement by evaluating the effects of HA, AB and MDA combination on mechanical and thermal behavior, biocompatibility and microbiological properties. Null hypotheses were that the addition of fillers and additive to traditional GICs does not change GIC 1) mechanical properties, 2) thermal properties, 3) cytotoxicity levels and 4) microbiological properties.

2. Materials and methods

2.1. Materials and sample preparation

A commercially available Glass Ionomer Cement (GIC) (Fuji II, GC, Tokyo, Japan) was selected as control. It was prepared by mixing the liquid (polyacrylic acid and water) with the powder, which contained fluoro-aluminosilicate glass, in a l:p ratio of 1:2.7, according to the manufacturer's instructions. The additives which were added to the base material are listed in Table 1.

Weight percentages were: 4 wt% for HA, 6 wt% for MDA and 1.5 wt % for AB. Samples with HA, MDA and AB in powder were labelled GIC-HA-MDA-ABp; samples with HA, MDA and AB in emulsion were labelled GIC-HA-MDA-ABe.

To prepare the experimental GIC groups, part of the powder was replaced by HA and AB powder and part of the liquid was replaced by MDA (which is in liquid form). In particular is preferable to consider MDA as an additive, becoming part of the cement matrix. AB was added as powder and as an emulsion to evaluate the effects of the formulations on antibacterial properties. When AB emulsion was used, part of the powder was replaced by HA powder and part of the liquid was replaced by MDA and AB. The liquid/powder weight ratio was kept as 1:2.7. Weighing scales with ± 0.1 mg precision (Mettler Toledo, type AB104-S, Greifensee, Switzerland) weighed each single component. The wet mixed paste was poured into silicon molds and left to air-dry for 6 h to remove samples from silicon molds without damaging macroscopically them. Silicon molds were closed to minimize humidity effect on mechanical behavior. Samples were then removed from molds and stored at room temperature (dry) or in water (wet) for 24 h until analysis. Mechanical tests, calorimetric analysis, morphological investigation, antibacterial and cell viability assays were conducted.

Analyses were performed after 24 h instead of the usual 7 days because good early-stage performance is needed to shorten clinical procedures and timing in non-collaborative patients.

2.2. Mechanical testing

Five samples of each material compressed after dry and wet storage followed by filter paper drying. A 600-grit sandpaper smoothed top and bottom surfaces. Samples were subjected to a compressive load (F) perpendicular to the top surface at a constant crosshead rate of 0.5 mm/min until failure occurred by using a dynamometer (LR30K, Lloyd Instruments Ltd., Fareham, UK) at room temperature (25 °C) as per ISO 9917-1 (2007).

The compressive strength of all specimens (σ_c) was calculated as follows:

$$\sigma_c = \frac{4F_{max}}{\pi d^2}$$

where F_{max} was the applied load at the maximum of the curve (N) and d is the sample diameter (mm) measured with a digital caliper (Mitutoyo, Tokyo, Japan) with 0.01 mm accuracy.

The elastic modulus (E_c) was calculated as follows:

$$E_c = F/\varepsilon A$$

where ε and A are the elastic strain (mm/mm) corresponding to load F and cross-sectional area of the sample respectively.

To evaluate absorbed elastic and plastic energy until sample failure, "toughness" or rather energy absorbed up to the maximum load was calculated from the stress-strain curves as the area under the curve up to Fmax. Since samples showed different behavior on the stress-strain curve, with some sliding at the end of the test, the mechanical performance of each sample was compared by taking the energy absorbed up to the point of maximum load.

2.3. Calorimetric analysis

To analyze the residual heat of each GIC sample, fractured samples were tested after setting. A differential scanning calorimeter (DSC) was used (Mettler-Toledo DSC 822E/400) with a heating cycle from 0 to 150 °C at 10 °C/min in a nitrogen atmosphere. Peak temperature and residual heat were obtained using the software STARe. The control GIC was also tested to compare the thermal behavior of modified GICs.

2.4. Scanning electron microscopy

After mechanical testing, samples were metallized with a thin layer of gold (15 nm, 99,99% of gold, $2 * 10–6$ Torr) in a thermal evaporator (Sistec thin film equipment model GP 20 by Kenosistec Angelantoni Group, Massa Martana (PG) - Italy). They were then analyzed by scanning electron microscopy (Field Emission Scanning Electron Microscope FESEM model SUPRA25, ZEISS, Oberkochen, Germany).

2.5. Microbiological testing

The antibacterial activity of each sample was assessed against *Streptococcus mutans* (ATCC 35668) from Thermo Fisher Diagnostics SpA, (Milan, Italy). A loopful of bacterial inoculum from the lyophilized culture was transferred to a Brain Heart Infusion broth (BHI; Thermo Fisher Diagnostics SpA) and incubated for 24 h at 37 °C. Bacterial growth was then assessed by turbidity in the broth.

Fresh *S. mutans* culture from turbid BHI broth was flood inoculated

Table 1
List of additives.

Abbreviation	Commercial name	Manufacturer
HA	Sealent®	Miromed S.r.l. Italy
MDA	Hepilor	Azienda Farmaceutica Italiana, Italy
AB	Ciproxin	Bayer S.p.A., Italy

HA: nanohydroxyapatite; AB: antibiotic; MDA: mucosal defensive agent.