JO JOURNAL OF OSSEOINTEGRATION periodontics and prosthodontics

Effects of bioactive additions on the physical properties of glass polyalkenoate cement

A. M. SALEM[1], A. S. QUTIESHAT[2]

[1] Dundee Dental School, University of Dundee, Dundee, UK
[2] Department of Conservative Dentistry, Jordan University of Science & Technology, Irbid, Jordan

TO CITE THIS ARTICLE
Salem AM, Qutieshat AS. Effects of bioactive additions on the physical properties of glass polyalkenoate cement. J Osseointegr 2019;11(3):497-503.

DOI 10.23805 /JO.2019.11.03.05

KEYWORD Biopolymer, Collagen, Glass polyalkenoate, Strength.

ABSTRACT

Aim Conventional glass ionomer cements are clinically attractive materials and have unique properties that make them useful dental restorative materials. The glass ionomer cements however are slightly brittle materials though they deform a little under load. They display high compressive strengths but slightly weak flexural strengths. Collagen type I and RGD peptides (Arg-Gly-Asp) are the most effective and widely used bioactive molecules to promote cell adhesion on a synthetic surface. This study investigates the effect of chairside addition of bioactive molecules (Collagen type I and RGD) into glass polyalkenoate cement on improving the physical properties.

Materials and methods Mechanical properties of the glass polyalkenoate cement (ChemFil Superior, Dentsply De Trey, Konstanz, Germany) were investigated both at baseline and after incorporating bioactive additions made at the time of mixing the material. The properties that are of potential significance for clinical durability were determined namely; compressive strength, diametral compressive strength, three-point flexural strength, diametral compressive fatigue limit, and biaxial flexural strength. Results: Additions of Type I Collagen and RGD to ChemFil Superior improved all physical properties measured except shear bond strength where no detriment was observed.

Conclusion Chairside additions of bioactive molecules to conventional glass ionomer restorations have potential clinical applications and represent a new paradigm in dentistry that can be utilized to improve biocompatibility, mechanical properties, and therefore, clinical durability. Improving the mechanical strength of glass ionomer restorations by optimized reinforcement strategy requires further investigation. Clinical significance: the methodology of mixing conventional glass ionomer with bioactive molecules for superior biocompatibility and reinforcement, developed in the present study, should be applicable to chairside dental procedures. The increase in physical properties of the glass polyalkenoate, achieved in the present study, may help extend its dental applications to the restoration of stress-bearing cavities.

INTRODUCTION

It is over 30 years since the glass ionomer cements were first introduced into clinical dentistry. The original glass ionomer cements did not receive widespread acceptance until the mid-1980s, especially in the United States (1). The clinical development and use of the glass ionomer cements was first explained by Mclean and Wilson (2-4), who were instrumental in the material development. However, these materials had some advantages such as ion exchange adhesion to the enamel and dentine, ability to release and uptake fluoride ions, and good thermal expansion and contraction (1). Original glass ionomer cements had low wear resistance, fractured easily, and required some protection to avoid over-hydration, it is likely that such problems accounted for the slow widespread acceptance of these materials.

Glass ionomer cements are moderately hard brittle materials, that display an ability to adhere to mineralized tooth structures (5). *In vitro* flexural or compressive strength testing of glass ionomer cements has been revealed to closely model the clinical loading situation (6). Under compressive stress the glass ionomer cements (ASPA) (167 N mm^{-2}) were considerably stronger than polycarboxylate cements (60-85 N mm^{-2}) but usually weaker than dental silicate cements (250 N mm^{-2}) (7). The tensile strength of early glass ionomer cements (ASPA) (10-17 N mm^{-2}) is higher than the tensile strength of polycarboxylate cements (6-12 N mm^{-2}), but is almost in the same range as the tensile strength of silicate cements (15 N mm^{-2}) (7).

The alteration in composition of the glass powder, by the incorporation of some metallic particles has been found to have some effects on the mechanical properties of glass ionomer cement (5). A group of researchers (8) compared the *in vitro* mechanical properties (compressive and diametral strengths in addition to tensile strength using the four-point test)

of metal reinforced and non–metal reinforced glass ionomer cements. It was found that the reinforced materials displayed significantly higher strengths than all the other materials though there was less difference in compressive strength.

More recently the effects upon compressive strength of adding both boric and phosphoric acids to conventional glass polyalkenoate have been investigated (9). The addition of 1% boric acid decreased the compressive strength, whereas the incorporation, of up to 2%, of phosphoric acid resulted in an increase in this property. This was attributed to increased cross-linking of the acid chains with aluminium.

It has been demonstrated that copolymers of acrylic acid and N Vinylpyrrolidone, with side chains of itaconic acid, improved the physical properties (compressive and diametral strength) by increasing the space available for ionic bond formation, with ions from the glass particles, and permitting more flexibility in the side chains (10). Concentration of these polymers was critical with detriment to properties if too high. The same workers also explored the synthesis and incorporation of nano-hydroxyapatite and fluorapatite particles into commercially available glass-ionomer powders and demonstrated better mechanical strength (11).

When applied to glass ionomer tethered amino-acid residues have the results of providing greater degrees of freedom for the pendant carboxylate ions to form salt bridges with the Ca2+ and Al3+ ions released from the glass particles (12).

It is well documented that the powder:liquid ratio at which the cement is mixed impacts on the physical properties (13). Encapsulation reduces the potential for such variation and results in better physical properties (14). In the case of hand-mixed glass ionomers, improvements in mixing characteristics result from granulation of glass particles at manufacture (15). This increases the wettability of particles upon exposure to polyacrylic acid and, from a health and safety perspective, reduces the potential for dust. In addition, such cements are easier to proportion as they adhere less to the manufacturers supplied proportioning spoon (15).

Apart from incorporation of fluoride for its therapeutic effect other agents have been examined. The conventional glass ionomer cements lend themselves to this as their hydrogel permits release and uptake of such agents and their setting reaction has no appreciable temperature rise to damage incorporated agents (16). The incorporation of oxalic acid into glass ionomers, with the aim of reducing dentine hypersensitivity by its release, accelerated the set of the material without affecting strength but, due to its low water solubility could only be introduced in low concentration (9). Another group of researchers examined the potential for chlorhexidine release from an experimental glass ionomer cement (17). This was with the intention of assessing the possibility of its incorporation and release due to its bactericidal effect. Their additions ranged from 0.5 to 13.0% by weight and in proportion to quantity added the working and setting times increased as the compressive strength decreased.

The incorporation of casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) into a self-cure glass ionomer was investigated (18). CPP-ACP agent inhibits demineralisation and favours remineralisation of tooth substance. They found that the incorporation of 3% CPP-ACP had the potential to improve the cements anti-cariogenic properties without adversely affecting its mechanical properties.

It has been demonstrated that the addition of a bioactive Sol-Gel glass (70SiO2.25CaO.5P2O5) to a commercial glass polyalkenoate (GC Fuji I cement) produced higher cell viability with no detrimental effect upon the diametral tensile strength (19). More recent work indicated that the inclusion of bioactive glass improves the biocompatibility of glass ionomer to fibroblasts (20).

In this vein, a group of researchers explored the effects of adding Type I collagen to promote the cellular adhesion, to glass polyalkenoate, of gingival fibroblasts (21). Although etching the surface of the material enhanced adhesion, the addition of Type I collagen to the cement significantly improved adhesion to these cells. This was not detrimental to the material's

ChemFil Superior		
Manufacturer	Dentsply Detrey GmbH 78467 Konstanz Germany	
Composition	Powder (1g)	Aluminium-sodium-calcium-fluoro-phosphoro-silicate (18:9:8:16:3:46) 0.84 g Polyacrylic acid (MW 30000-45000) 0.15 g
	Liquid	Distilled/deionized water
Colour	L2	
Batch number	1110001332	
Date derived from manufacturer material safety data sheet		

TABLE 1 Details of the glass polyalkenoate cement studied.

compressive strength; indeed, it improved it, providing the addition did not exceed 0.01% collagen.
Previous works (22), utilising immunocytochemistry and western blotting, concluded that the addition of RGD and Type I collagen to ChemFil Superior enhanced the expression of vimentin, indicating that the cells have become more fibroblastic in nature. Such additions have the potential to promote cellular attachment to glass polyalkenoate cement restorations. However, the influence of the biological content on the material's physical properties remains to be investigated.
This work sought to investigate the effect of incorporating bioactive additions into glass polyalkenoate cement on improving the physical properties.

MATERIALS AND METHODS

The compositional details of ChemFil Superior are summarised in Table 1.
The work reported here is an examination of the potential of chairside addition of bioactive molecules, made at the time of mixing this material.
Unless otherwise stated, GraphPad PRISM software (version 5.0, GraphPad Software Inc, San Diego, California, USA) was used for all statistical analysis. Statistical significance was signified at $P < 0.05$.
At baseline and following the additions (additions identified in previous work as having potential for cellular attachment) (22) the following physical properties, of potential significance for clinical durability, were determined.

- Compressive strength (15 specimens).
- Diametral compressive strength (15 specimens).
- Three-point flexural strength (15 specimens).
- Diametral compressive fatigue limit (15 specimens).
- Biaxial flexural strength (10 specimens).

Fabrication of specimens

A variety of moulds were used to manufacture the specimens. In the case of flexural specimens, a sectional Perspex mould, giving a specimen size of 25 x 2 x 2 mm was used. For the specimens tested for compressive strength, diametral compressive strength and diametral compressive fatigue a split stainless-steel mould, giving specimens' sizes of 6 mm long and 4 mm diameter was used.
Biaxial flexural strength specimens were fabricated in silicone rubber moulds giving specimen sizes of 2 mm thick x 12 mm diameter. None of the moulds was vaselined, to facilitate specimen release, other than the stainless-steel compressive mould.
Prior to mixing the cements under test the compressive strength mould was placed upon a flat glass slab covered by a clear cellulose matrix strip (Hawe-Neos Dental, Bioggio Switzerland). In all other cases the mould designs themselves contained a flat base against which the base of the specimen was formed. Thereafter the mixed cement was applied into the well of the moulds using a plastic spatula, with packing action, to slight excess. A cellulose matrix strip was then applied to the exposed surface and pressure applied to the material through a flat glass slab on which was placed a 5 Kg weight for 5 minutes. Once this time had elapsed the specimen was removed from the mould. If upon visual inspection no defects were found the specimen was accepted for storage and testing.
Baseline specimens were prepared according to the manufacturer's instructions. Specimens to receive bioactive additions, were dispersed according to the manufacturer's instructions at a powder: liquid ratio of 1:1; the additions investigated were.

- Collagen type I 0.1% (100µg/ml) (prepared in house)(23).
- RGD (5mg/ml) (Sigma-Aldrich, St. Louis, USA).

Bioactive additives and their concentrations were chosen with reference to previously conducted cellular studies (22,24); as such additions have shown to offer great potential to foster cellular interaction. Once fabricated all specimens were stored in distilled water at 37 °C for one week prior to testing.
An Instron Universal testing machine (Model 4469, Instron Ltd., High Wycombe, UK) was used to perform all tests unless stated otherwise. The testing procedures described in this work utilised standard methodology used in the laboratories where the tests were undertaken.
Compressive and Diametral tensile strength were determined at a cross head speed of 1 mm min. Prior to testing the length and diameter of each specimen was measured using a micrometer. The results for each material were expressed as a mean and standard deviation. This data was subject to analysis of variance with post hoc testing using the Tukey comparison of means test.
The formula used to determine compressive strength was (equation 1):

$$\text{Compressive Strength (MPa)} = \frac{\text{Force at failure in Newtons}}{\text{Cross Sectional area of specimen}} \quad (1)$$

The formula used to determine compressive strength was (equation 2):

$$\text{Diametralx compressive Strength (MPa)} = \frac{2F}{\pi DT} \quad (2)$$

Where F is the force (N) at failure, D is the specimen diameter (mm) and T is its length.

Three-point flexural strength was determined at a cross head speed of 1 mm/min. Prior to testing the breadth and depth of each specimen was measured using a

micrometer. A support separation distance of 20 mm was used for this test. The results for each material were expressed as a mean and standard deviation. This data was subject to analysis of Variance with post hoc testing using the Tukey comparison of means test.
The formula used to determine flexural strength was (equation 3):

$$\text{Flexural strength (MPa)} = \frac{3FL}{2bd^2} \quad (3)$$

Where F is the force (N) at failure, L is the support separation distance in mm, b is the specimen width in mm and d is its depth in mm.
Diametral tensile fatigue limit was determined at a cross head speed of 10 mm/min' under load control. Each specimen was subjected to cyclic diametral compressive loading to failure or survival at 1500 cycles. At the commencement of the test a load of 2 Newton's was applied to the specimen before the load was increased to the maximum applied load for the test. Two Newton's was therefore the minimum force applied to each specimen.
This was undertaken to minimize the effect of any bounce of the specimen upon the platen of the testing machine that could occur if the minimum load was zero Newton's. The starting level was the applied load necessary to achieve 60% of the measured diametral compressive strength at 10 mm min. Testing and fatigue limit calculation followed the staircase method described by Draughn (25). Where a specimen survived the next test, maximum load was increased by a predetermined load increment and where it failed the load was decreased by the same increment. Upon completion of the test, after typically 15 specimens had been tested, analysis of the data was based upon the least frequent event (failure or survival). Standard deviation of this property was calculated according to the procedures advocated by Dixon and Mood (26) and Draughn (25).
Biaxial flexural strength: The Universal Testing Machine was used to determine the bi-axial flexural strength of the discs. Each disc was placed on three circumferentially arranged fixed ball bearings spaced every 120" around the perimeter of a circle of radius 4 mm on a specially constructed jig. Load, to fracture, was applied perpendicular to the specimen's surface, at its centre, through a stainless-steel rod of radius 1 mm at the point of specimen contact. The load at fracture was recorded and for each specimen bi-axial flexural strength was calculated using the following formulae (equation 4) as reported by Shetty et al. (27).

$$6 = \frac{AP}{t^2}$$
$$A = 3/(4\pi)(2(1+\nu)\ln(a/rxo) + (1-\nu)\left\{\frac{2a^2 - ro^{x2}}{2b^2}\right\} + 1 + \nu \quad (4)$$

Where P is the maximum at failure, v is Poisson's ratio, a is the radius of the support circle (4 mm), b is the radius of disc specimen (6 mm), t is the thickness of the disc specimen (2mm) and r is the radius of the ball used on the loading surface.
For small rxo values such as that in the study (equation 5):

$$r_0^x = \sqrt{1.6\, r_0^{x2} + t^2} - 0.675\, t \quad (5)$$

Where r is an equivalent radius of contact between the loading ball and disc specimen, where loading can be considered to be uniform. The strength values were calculated using a Poisson's ratio 0.35. This value has been recommended to apply to the materials of the type tested (28).

RESULTS

Table 2 summarises the observed mean values and their standard deviations for all properties determined for Chemfil Superior with and without the addition of RGD and Type 1 collagen.
For each property analysis of variance with post testing by the Tukey comparison of means test demonstrated statistically significant improvements in property values following addition of RGD and Type 1 collagen for compressive strength (P<0.001),

Property/Material State (MPa)	ChemFil Superior	ChemFil Superior plus RGD	ChemFil Superior plus Type I collagen
Compressive strength	50.0 (19.0)	93.0 (30.0)	101.0 (25.0)
Diametral tensile strength	6.6 (2.1)	11.0 (2.7)	10.2 (2.3)
Three-point flexural strength	11.4 (3.8) n=16	16.4 (5.7)	12.6 (2.8)
Diametral tensile fatigue	2.2 (1.2)	6.9 (0.6)	4.7 (0.1)
Biaxial flexural strength	21.8 (7.0) n=10	31.7 (5.6) n=10	34.9 (5.5) n=10
Number of specimens (n) per test is 15 unless otherwise stated.			

TABLE 2 Summary of mean values, standard deviations and number of specimens tested of ChemFil Superior with and without addition of RGD and Type I collagen.

diametral compressive (P<0.001) and biaxial flexural strength (P<0.01 for RGD and P<0.001 for Type 1 collagen additions respectively). In the case of 3 point flexural strength only the addition of RGD conferred a significant increase in this property (P<0.01). Student's t tests demonstrated significantly improved diametral tensile fatigue limits following addition of RGD and Type 1 collagen to Chemfil Superior (P<0.001).

DISCUSSION

This laboratory work sought to investigate the effect of incorporating bioactive additions into glass polyalkenoate cement on improving the physical properties of the cement. Baseline property values were determined of the unmodified material to establish if the modifications had an effect upon material properties. Conventional glass ionomer (ChemFil Superior) in freeze dried form was selected to be modified. It was chosen because ChemFil Superior is presented in powder and liquid format for hand mixing, chemically set, resin-free, and involves no light curing. It was favoured over encapsulated materials as it more readily permitted modification of their constituents by the inclusion of bioactive additives.
It is important to note that the properties evaluated were those thought to be most relevant to clinical success. Although not reported here many attempts were made to obtain a value of flexural fatigue strength for ChemFil Superior. Due however to the often premature failures of ChemFil superior specimens, not even making one flexural fatigue cycle, flexural fatigue was not tested. A search of the literature failed to identify any studies that report flexural fatigue properties of ChemFil superior perhaps reinforcing the difficulties experienced.

Compressive strengths: longitudinal and diametral

Both longitudinal and diametral compressive strength testing of specimens was undertaken using an Instron universal testing machine at 1mm min. As observed by others, the ends of longitudinal compressive specimens must be lapped flat, otherwise edge effects lead to premature failure of the specimen by facilitating crack initiation and propagation (29). Practically, however, it is not possible to precisely lap flat and so in the present investigation this was not carried out. To therefore obtain a more meaningful assessment a tensile strength test was also performed. This test avoids edge effects and is the test of choice for brittle materials as used in this study (30). Although the baseline results obtained in this study are in the ballpark of values published in the literature, they are lower. This is in all likelihood due to the clinically realistic mixing and storage regimes. Both of these properties are considered to be of relevance to clinical function (31). Mastication applies compressive forces as tested here by the compressive strength, whereas the diametral compressive test replicates in some way clinical failures due to tensile stress (32). In this work convention has been broken for the term compressive diametral has been used to indicate the experimental set up and direction of applied force. Conventionally such a test is called a diametral tensile test for the compression plates, either side of the specimen; apply largely tensile forces with also an element of compression. The latter has been identified as a factor that to a degree prevents the propagation of the tensile crack (30). True tensile testing of brittle materials, such as glass ionomer, is not practicable and was internationally agreed some time ago to therefore adopt the diametral tensile strength test as a means of assessing this property (33).

Three-point flexural strength

the jig used to determine the three-point flexural strength of the materials tested contained within it two cylindrical rollers upon which the specimens sat. This was preferable to point contacts, for these risked creating points of stress concentrations which would enhance crack propagation. Karbhari and Wang (34)commented that the ratio of support separation to the specimen depth should be 10 in order to ensure that bending moments dominate over shear forces, this practice, and the experimental set up conform to ISO 99.17 (35]. This was achieved by this experimental set up. The baseline values obtained are similar to other comparable materials in the literature.

Diametral compressive fatigue

Although this work has examined many static properties, it should not be forgotten that in its lifetime a dental restoration will be subjected to many cyclical forces. These cumulatively can cause a material to fail. It is therefore potentially misleading to report static properties alone. It was for this reason that the tensile fatigue limit of the materials alone and with additives was determined. In order to minimise edge effects, this property was favoured over compressive fatigue and for practical reasons the flexural fatigue was not determined. The method used to determine this property is widely accepted and seeks to relate the number of cycles to failure versus the applied stress (25). The definition of the number of cycles to be survived was set empirically at 1500 cycles, so that work was achievable in a realistic laboratory time frame. As all materials tested followed the same testing regimen, this permitted ready comparison. Surprisingly, a search of the literature revealed no diametral fatigue limits against which the present values can be compared.
When the fatigue limits were compared to the mean diametral tensile strength values, they are lower than it would be expected. The biological additions, however, significantly increased the fatigue strength of ChemFil superior.

Biaxial flxural strength

The materials investigated in this study are brittle. By their nature they contain flaws and such inclusions are

exacerbated by handling and mixing. The impact of these upon the physical property of biaxial flexural strength is a useful test, for it is said to be more searching for defects than a uni-axial test (36). The testing geometry used in this study, comprising a circular disc supported upon three balls, has been shown to be:

- insensitive for a specimen whose surface is rough.
- insensitive to edge effects that could act as stress concentration precipitating early failure (36).

The calculation uses the Poisson's Ratio. This is defined as the ratio of lateral strain to axial strain in an axially loaded specimen. It has been determined previously by Akinmade and Nicholson (28) as 0.35 for glass ionomer, the value used here, though in a subsequent paper they quote a value of 0.27 (28). This work treats all results the same way and so this difference in terms of relative performance is of no relevance. The baseline values obtained for biaxial flexural strength in this work are in good agreement to those reported by other researchers (37).

The mechanism of effect

It has been demonstrated that a setting glass polyalkenoate cement would undergo a pH drift over a 24-hour period ranging from 2.2 up to 6.2 (38). This range of acidity can favourably induce collagen type I to facilitate the formation of molecular aggregates, fibrils and eventually fibers (39). Collagen granules have been previously observed to form on glass ionomer surfaces using SEM and AFM analyses (40). Apart from the role of these formations in facilitating cellular attachment, they can reinforce the material itself. Some indirect evidence supporting this hypothesis is demonstrated herein by the improvement in physical properties when collagen type I was added.

As regards the positive effect of RGD addition to the glass polyalkenoate cement, a clear mechanism has not been identified in this work. It is however possible that RGD's amino acids can form ionic bridges that crosslink with Ca+2 and Al+3 ions of the glass ionomer cement. Hydrogen bonding and dipole-dipole interactions with the material's parent acid may also be a factor to consider in this respect. Moreover, given their molecular structure, non-bonded interactions may be a possibility. A group of researchers demonstrated that the addition of RGD-peptide-incorporated chitosan fibers to a pure calcium phosphate cement improved by three-fold its mechanical strength through a fiber reinforcement mechanism (41). Their findings may give some support to the mechanism of effect proposed here. However, further work via electron scan microscopic analyses is required to more fully understand the mechanism of Type I collagen and RGD interaction with glass ionomer.

CONCLUSIONS

With the exception of three-point flexural strength all the properties tested improved significantly, compared to the baseline values, for the samples of ChemFil Superior to which 0.1% collagen Type I was added. As regards additions of RGD similar improvements were observed for all properties.

Chairside bioactive additions to conventional glass ionomer restorations have potential clinical applications and represent a new paradigm in dentistry that can be used to improve biocompatibility, mechanical properties, and therefore, longevity. Improving the mechanical strength of glass ionomer restorations by optimized reinforcement strategy requires further investigation.

Clinical significance: the methodology of mixing conventional glass ionomer with bioactive molecules for superior biocompatibility and reinforcement, developed in the present study, should be applicable to chairside dental procedures. The increase in physical properties of the glass polyalkenoate, achieved in the present study, may help extend its dental applications to the restoration of stress-bearing cavities.

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