Research Article

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Glass ionomer bone cements based on magnesium-containing bioactive glasses

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Abstract: Glass ionomer cements (GIC) are used in restorative dentistry and their properties (low heat during setting, adhesion to mineralised tissue and surgical metals) make them of great interest for bone applications. However, dental GIC are based on aluminium-containing glasses, and the resulting release of aluminium ions from the cements needs to be avoided for applications as bone cements. Replacing aluminium ions in glasses for use in glass ionomer cements is challenging, as aluminium ions play a critical role in the required glass degradation by acid attack as well as in GIC mechanical stability. Magnesium ions have been used as an alternative for aluminium in the glass component, but so far no systematic study has looked into the actual role of magnesium ions. The aim of the present study is therefore the systematic comparison of the effect of magnesium ions compared to calcium ions in GIC glasses. It is shown that by partially substituting MgO for CaO in simple SiO_2 -CaO- CaF_2 glasses, ion release from the glass and, subsequently, GIC setting behaviour can be adjusted. Magnesium ions act as typical network modifiers here but owing to their larger field strength compared to calcium ions reduce ion release from the glasses significantly. By choosing an optimum ratio of magnesium and calcium ions in the glass, GIC setting and subsequently compressive strength can be controlled.

Keywords: bioactive glasses; magnesium; dissolution; continuous flow; glass ionomer cement

1 Introduction

Cements for prosthetic stabilisation or spinal corrective surgeries (vertebroplasty, kyphoplasty) typically consist of polymethylmethacrylate [1, 2]. They exhibit a number of drawbacks which include high curing temperatures or the presence of unreacted and toxic methacrylic acid monomers. They also do not bind to bone and are held in place by mechanical interlocking only [2–6]. As a result, there is a demand for alternative non-toxic cements with bone bonding capability.

Glass ionomer cements (GIC) have been used in restorative dentistry as filler or luting materials for decades [7, 8]. They are formed by an acid-base reaction between a polymeric acid and an acid-degradable fluoro-aluminosilicate glass [9]. Owing to their direct adhesion to mineralised tissue these cements have aroused interest as bone cements. However, while Al^{3+} ions play a key role in cement setting, stability and performance, they are known to be neurotoxic [10] and impede bone mineralisation [11–13]. Bioactive glasses may present an alternative to these aluminosilicate glasses owing to their fast ion release when in contact with aqueous solutions [14–16].

Efforts to substitute Al^{3+} ions with other multivalent ions, e.g. $\text{Fe}^{2+/3+}$ or Zn^{2+} , have already been reported but the resulting cements exhibited drawbacks regarding cement stability or even cell toxicity [17–19]. By contrast, studies showed that GIC formed using Mg-containing glasses revealed more promising properties [17, 20, 21]. Magnesium is an important element found e.g. in bone apatite. In addition, it is necessary in preventing osteoporosis [22, 23].

The aim of this study was to investigate the effect of MgO for CaO substitution on glass properties and particularly on GIC formation and properties.

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2 Materials and methods

2.1 Glass synthesis and basic characterisation

A series of glasses in the system SiO_2 -CaO-MgO- CaF_2 was prepared via a melt-quench route by replacing one third (glass 15Mg) or two thirds (glass 30Mg) of CaO with MgO (Table 1). Mixtures of SiO_2 , CaCO_3 , CaF_2 and MgCO_3 were sintered together in a platinum crucible at 1400°C using an inductively heated furnace and afterwards melted at 1500°C for 1 hour. To prevent crystallisation the melts were rapidly quenched in water. Glass powders were prepared using ball milling (KM1, Janetzki, milling time 30 min) and sieving with analytical sieves to obtain fine ($\leq 38\ \mu\text{m}$) or coarse ($38\ \mu\text{m} \leq x \leq 63\ \mu\text{m}$) powders.

Glass monoliths were prepared by re-melting the glass frits at 1500°C . The melt was then cast into brass moulds, which for annealing were placed into a pre-heated furnace set to 30 K below the glass transition temperature (T_g). Glasses were cooled down to room temperature in the switched-off furnace overnight. Compositions of prepared glasses were analysed using energy-dispersive X-ray spectroscopy (EDX; JOEL JSM-7001F scanning electron microscope equipped with an EDAX Trident analysing system) on polished monoliths. Glass density was measured using helium pycnometry (AccuPyc 1330-1000, Micromeritics GmbH). Furthermore, the molar volume (V_m) of the glass series was calculated as described previously [24]. Glass powders were characterised by powder X-ray diffraction (XRD; D5000, Siemens) and attenuated total reflectance Fourier transform infrared spectroscopy (ATR-FTIR; Frontier IR/NIR, Perkin Elmer; ATR sample holder, Specac). In addition, particle size distribution of fine and coarse powder was determined by dynamic light scattering (DLS; Malvern Mastersizer 2000 equipped with HYDRO 2000S module).

2.2 Glass thermal analysis and crystallisation

Glass transition temperature (T_g) and crystallisation peak temperature (T_x) were determined by differential thermal analysis (DTA; in-house made device, heating rate: $10\ \text{K min}^{-1}$) using fine glass powder.

Dilatometry (DIL 402 PC, Netzsch, heating rate: $5\ \text{K min}^{-1}$; measurements performed on glass rods 5 mm in diameter and 20 mm in length) was used to determine T_g ,

the dilatometric softening point (T_d) and the thermal expansion coefficient (α ; temperature range 100 to 400°C).

Fine glass particles were heated in a furnace (LT3/11/B410, Nabertherm GmbH, Lilienthal, Germany) to T_x , and the temperature was held constant for either 10 min or 3 hours before switching off the furnace and leaving the samples to cool down in the furnace over night. Samples were analysed by powder X-ray diffraction (Miniflex 300, Rigaku Corporation, Tokio, Japan; $\text{Cu K}\alpha$, 30 kV, 20 mA, $5\text{--}75^\circ 2\theta$).

Table 1: Nominal and analysed (in brackets) glass composition (mol%).

	SiO_2	CaO	MgO	CaF_2
0Mg	45 (42.05 ± 1.54)	45 (47.18 ± 3.46)	0 -	10 (10.77 ± 1.41)
15Mg	45 (43.76 ± 1.53)	30 (31.55 ± 2.42)	15 (15.27 ± 0.76)	10 (9.41 ± 1.14)
30Mg	45 (43.64 ± 1.59)	15 (16.79 ± 2.38)	30 (29.09 ± 1.32)	10 (10.45 ± 1.32)

2.3 Static and dynamic dissolution experiments

For static dissolution tests 75 mg of coarse glass powder was immersed in 50 mL cell culture medium (Minimum Essential Medium Eagle M4526, Sigma-Aldrich) at 37°C for either 24 hours or 7 days. Glass powders were analysed using ATR-FTIR after each immersion time point.

Early stage ion release was investigated by dynamic dissolution experiments, where a dynamic flow cell was connected to an inductively coupled plasma optical emission spectrometer (Optima 5300 DV, Perkin Elmer) as described previously [25–27]. The flow cell contained glass powder (200 to 245 mg; normalised to 243 mg) with a grain size between 315 and $500\ \mu\text{m}$. Random packing was assumed. On-line measurement and experimental set-up are shown in detail elsewhere [25, 27, 28].

2.4 GIC preparation

GICs were prepared by weighing and hand mixing glass powder and poly(vinylphosphonic acid-co-acrylic acid)

(PVPA-co-PAA; 40 wt% copolymer solution, weight average of chain length between 40 and 70 kDa according to manufacturer, First Scientific Dental GmbH). A glass to polymer ratio of 1:3 by weight was used to form cements. The amount of Mg containing glasses was adjusted owing to lower molecular weight of Mg compared to Ca. After mixing, the cement was filled into PTFE moulds (7 mm in height, 4 mm in diameter) and set for 1 hour at 37°C. Samples were stored for up to 28 days either inside the mould, in deionised water or in 100% relative humidity (rH) as described elsewhere [21].

2.5 GIC setting rate and mechanical testing

Changes in cement structure during setting were analysed using ATR-FTIR. Spectra were acquired every 60 seconds for the first 10 minutes, every 150 seconds for the following 10 minutes and one spectrum at 25 minutes. The ratio of the peak heights of the carboxylate band at 1550 cm⁻¹ and the carboxyl band at 1700 cm⁻¹ were calculated to characterise the setting rate of the different cement compositions [29–33].

For mechanical testing, specimens were stored for 1 day either inside the PTFE mould at 37°C or for 1 hour inside the mould followed by 23 hours in either deionised water or at 100% rH (both at 37°C). For longer term studies, after storage in the PTFE mould for 1 hour, samples were stored in deionised water at 37°C for either 6 days 23 hours (“7 days”) or 27 days 23 hours (“28 days”) before mechanical testing. GIC specimens for mechanical testing were prepared using fine or coarse powder as described above. Compressive strength was measured using a universal testing machine (Z005, Zwick/Roell) with a cross-head speed of 1 mm min⁻¹. Kolmogorov-Smirnov test ($p = 0.05$) was performed on compressive strength results to test for normal distribution of the data. Results were analysed by one-way ANOVA followed by Tukey test ($p < 0.05$; $n = 8$).

3 Results

3.1 Glass formation

Prepared glasses (monoliths and frit) were clear, and XRD patterns only showed amorphous halos indicating the amorphous state of the glasses (results not shown). Results of EDX analyses of different glass compositions are shown in Table 1. No significant amounts of fluoride were

lost during melting. Substitution of MgO for CaO resulted in a decrease in density and molar volume (Figure 1).

Figure 1: Density and molar volume (V_m , blue, right axis) of the glasses.

Results of particle size distribution measurements for fine and coarse glass powder are shown in Table 2. No significant variation with glass composition was observed.

Table 2: Glass particle size distribution: cumulative volume percentage (D10, D50, D90, in μm) for fine and coarse powder.

Glass		D10	D50	D90
0Mg	fine	2.4	13.3	30.7
	coarse	8.8	31.4	81.8
15Mg	fine	1.8	11.1	27.1
	coarse	4.1	34.5	79.5
30Mg	fine	2.0	11.7	28.1
	coarse	4.1	34.5	79.5

3.2 Thermal analysis and crystallisation

The DTA curve (Figure 2a) of the Mg-free glass MgO showed two overlapping broad crystallisation peaks (T_x) between 800 and 850°C. With increasing MgO substitution, T_x moved to lower temperatures (Figure 2b), the second crystallisation peak became narrower and increased significantly in intensity. Parallel to that, the intensity of the first crystallisation peak decreased markedly. The two Mg-containing glasses showed liquidus temperatures between 1000 and 1100°C, while no such effect was observed for glass 0Mg in the temperature range studied here. Liq-

Figure 2: Thermal analysis: (a) DTA curves and (b) T_g and T_d determined by dilatometry as well as T_g and T_x (blue, right axis) determined by DTA. Lines are visual guides only.

uidus temperature also shifted to lower temperatures with increasing MgO substitution.

Glass transition temperature (T_g) went through a minimum with increasing MgO substitution. From 0Mg to 15Mg T_g decreased from 655°C to 620°C, while a further increase in MgO substitution resulted in a T_g of 638°C (glass 30Mg; Figure 2b). The dilatometric softening point (T_d), by contrast, increased with increasing MgO substitution, while the thermal expansion coefficient showed non-continuous change: 0Mg had an α of $(10.24 \pm 0.01) \times 10^{-6} \text{ K}^{-1}$, for 15Mg it was $(11.01 \pm 0.01) \times 10^{-6} \text{ K}^{-1}$ and for 30Mg $(9.06 \pm 0.01) \times 10^{-6} \text{ K}^{-1}$.

Heat treatment at T_x for 10 minutes resulted in crystallisation of glasses 0Mg and 15Mg, with cuspidine ($\text{Ca}_4\text{Si}_2\text{O}_7\text{F}_2$) being the main crystal phase (Figure 3). Results for glass 30Mg, by contrast, did not show any presence of crystalline phases. Longer heat treatment for 3 hours resulted in additional phases appearing for glasses 0Mg (wollastonite, CaSiO_3) and 15Mg (diopside, $\text{CaMgSi}_2\text{O}_6$) while glass 30Mg crystallised to diopside.

3.3 Static dissolution experiments

Glass powders were stored in phosphate-containing cell culture medium to investigate potential apatite formation. ATR-FTIR spectra (Figure 4) of untreated glasses showed a non-bridging oxygen band (930 cm^{-1}) as well as a lower intensity bridging oxygen band (1040 cm^{-1}) [34]. The intensity of the non-bridging oxygen band decreased and disappeared almost completely with immersion time in the culture medium. A broad shoulder appeared between

560 and 600 cm^{-1} at 1 day for all glasses, indicating the formation of an amorphous calcium phosphate surface layer [35]. At 7 days a broad PO vibration band appeared around 1100 cm^{-1} [36]. The characteristic split band for crystalline calcium orthophosphates at 560 and 600 cm^{-1} were not detected in the time frame studied here [37].

3.4 Dynamic dissolution experiments

Results of dynamic dissolution experiments (Figure 5) were normalised to the amount of each ion present in the untreated glass. While the modifier ion release ($\text{Ca} + \text{Mg}$) was comparable for glasses 0Mg and 15Mg (Figure 5a,b), it decreased dramatically for higher MgO substitution, *i.e.* for glass 30Mg (Figure 5c). The concentration of silicon species in solution, however, decreased continuously with MgO substitution in the glass.

3.5 GIC setting behaviour

Changes in the chemical structure of the cements during setting were followed by ATR-FTIR (Figure 6). As an example, spectra of the cement formed from 15Mg and PVPA-co-PAA are shown in Figure 6a. Over time, the band corresponding to carboxyl groups (COOH ; 1700 cm^{-1}) decreased significantly in intensity whereas the intensity of the carboxylate band (COO^- ; 1700 cm^{-1}) increased. Calculating the ratio of peak heights of these two bands (COO^-/COOH , Figure 6b) can provide information about the setting rate of the cements [30]. Cements formed from glass 0Mg showed the steepest slope at early time points (up to about 3 min),

Figure 3: XRD patterns of glasses treated at T_x for (a) 10 minutes or (b) 3 hours.

Figure 4: FTIR spectra for untreated glasses (dotted line) and for glasses stored in cell culture medium for 1 day (dashed line) or 7 days (continuous line).

while with increasing MgO substitution the slope became less steep. This indicates a slower conversion from COOH to COO^- , *i.e.* a slower increase in ratio COO^-/COOH . In addition, changing the glass composition also delayed the time until the ratio of COO^-/COOH bands reached a constant value. However, by about 25 min all GIC showed a ratio of about 2.5.

3.6 GIC mechanical properties

Compressive strength measurements showed normal distribution. Results are presented as box-whisker plots to show a comprehensive overview of the data distribution, *i.e.* median, interquartile data range (corresponding to data points between the 25% and 75% quartile), mean and

outliers. The whiskers represent 1.5 times the interquartile range or the maximum or minimum values if they are lying within this range.

Mechanical testing (Figure 7) revealed an influence of glass composition on GIC compressive strength. Cements prepared from glass 15Mg gave significantly higher compressive strengths than those prepared from either glass 0Mg or glass 30Mg. Results for cements prepared from glasses 0Mg and 30Mg were comparable. This trend was observed for all storage conditions studied here.

A reduction in glass particle size led to an increase in compressive strength for all cements (Figure 7a). Maximum compressive strength obtained for GIC increased from 5.28 to 7.59 MPa (Mg0), from 9 to 11.51 MPa (Mg15) and from 5.04 to 6.8 MPa (Mg30). Variation in storage time in deionised water showed no significant changes in compressive strength between 1, 7 or 28 days for any of the cements (Figure 7b).

The effect of variations in storage medium is shown in Figure 7c; all cements exhibited the same trend of compressive strength with variation of storage medium: highest strengths were obtained if samples were stored inside the mould only, without getting into contact with humidity or water. Maximum compressive strengths of the GIC prepared from Mg0, Mg15 or Mg30 were 11.58, 20.62 and 13.77 MPa, respectively, after storage in the mould only. By comparison, storing the specimens at 100% relative humidity significantly reduced compressive strength. Maximum compressive strength of cements stored at 100% relative humidity decreased to 9.69, 12.22 and 7.19 MPa with increasing MgO content. Storing the specimens in deionised water led to a further decrease in compressive strength.

Figure 5: Normalised ion concentrations in dynamic dissolution experiments using glasses (a) 0Mg, (b) 15Mg and (c) 30Mg in Tris buffer.

Figure 6: (a) Time-dependent FTIR spectra for the 15Mg-PVPA-co-PAA cement (* normalised to the CH bending vibration at 1454 cm^{-1} [38]); (b) setting profile of all three GICs.

4 Discussion

Commercially available GIC are formed by mixing a polymeric acid and a fluoro-aluminosilicate glass. As aluminium ions are neurotoxic [10] and negatively effect bone mineralisation [11–13], the use of aluminium-containing GIC as bone cements is not recommended, and new glass compositions are desired. Bioactive glasses are a potential alternative. Larry L. Hench invented these glasses almost 50 years ago [39]. Since then, these glasses have been used as implants for bone regeneration in generations of patients. In the last decades, many new bioactive glass compositions have been investigated [17–19]. In the present study, a simple ternary glass ($\text{SiO}_2\text{-CaO-CaF}_2$) is investigated for use in GICs, and the effect of MgO for CaO substitution on glass and GIC properties was investigated.

4.1 Glasses

Owing to the lower atomic weight of Mg compared to Ca, glass density decreased with increasing MgO for CaO substitution. The smaller ionic radius of Mg^{2+} compared to Ca^{2+} resulted in a decrease in molar volume with increasing MgO substitution, corresponding to an increasing compactness of the glass network. For the same reason, MgO bonds are typically shorter (1.97 Å [40]) than CaO bonds (2.38 Å).

Glass thermal properties were influenced by the glass composition in a non-linear way, as T_g of glass 15Mg was lower than T_g of glass 0Mg and 30Mg. This is in contrast to previous studies, which showed a decrease in T_g with increasing MgO for CaO substitution in bioactive glasses [41–44]. One reason for this difference in thermal behaviour may be that the present glass system contains a significant amount of fluoride and partitioning of the metal cations between the silicate and the fluoride phase [45] may account for these non-continuous changes in T_g . While the mixed alkali effect is well known to cause ther-

Figure 7: GIC compressive strength: (a) comparison of GIC (stored for 1 day in deionised water at 37°C) prepared using coarse or fine powder, (b) effect of storage time in deionised water on GIC prepared from coarse powder, (c) effect of storage medium on GIC stored for 24 hours at 37°C. Outliers are presented as crosses, mean values as small squares. Compressive strength of samples labelled with the same symbol were not significantly different, $p = 0.05$. All other samples were significantly different, $p < 0.05$.

mal properties to go through minima or maxima [46, 47], a mixed alkaline earth effect as recently been observed in magnesium-containing aluminosilicate glasses [48] and may possibly be the cause of the minimum trend observed for T_g in the present study. A similar effect has also been observed for magnesium-containing phosphate glasses [49]. By contrast, T_d increased continuously with increasing MgO substitution and did not show indications for a mixed cation effect. This trend for T_d could be caused by the higher field strength of Mg^{2+} compared to Ca^{2+} , but we cannot currently explain why an increasing MgO substitution had such different effects for T_g and T_d .

Glass crystallisation temperature showed a different trend again and decreased with increasing MgO for CaO substitution. This trend is in contrast to previous observations, where an increase in T_x was observed with increas-

ing MgO for CaO substitution [41, 42] and explained by the higher field strength of Mg^{2+} compared to Ca^{2+} . The DTA traces show, that not only the maximum temperature of the crystallisation peak changes but also its intensity and broadness. A broad crystallisation peak is often taken as an indication for surface crystallisation, while a narrow one suggests bulk crystallisation [50]. This suggests that with increasing MgO for CaO substitution the nucleation and crystallisation process changes for surface to bulk, although further studies are necessary to make any clear statements as to the crystallisation mechanism. Despite DTA results indicating a decrease in T_x with increasing Mg content in the glass, composition 30Mg seemed to have a lower tendency to undergo crystallisation than the other two glasses. However, as with increasing Mg content in the glass the crystal phases change from cuspidine + wol-

lastonite (0Mg) to cuspidine + diopside (15Mg) to diopside (30Mg), this change in crystal phases forming may explain some of the observed effects including the decrease in T_x or the shape of DTA traces.

The thermal expansion coefficient went through a maximum for intermediate MgO substitution (glass 15Mg), which, like the minimum trend of T_g , suggests a possible mixed cation effect. However, partitioning of modifier cations between silicate rich phases and fluoride rich phases of the glass network may well account for this trend.

Dynamic dissolution experiments give insight into ion release from the glasses. As ion release is important for GIC setting, owing to poly(vinylphosphonate-co-acrylate) chains being cross-linked by metal cations, ion release studies may help to understand differences in cement setting with variation in glass composition.

The shape of the release profiles is similar for all glasses, with ion concentrations increasing very fast over the first 1 to 2 minutes, and concentrations remaining relatively stable afterwards. This suggests that both Ca and Mg act as typical modifiers in the glass, behaving in a similar manner. This is in agreement with previous studies on Mg-substituted bioactive glass 45S5 [28, 42, 51], but is in stark contrast to one study suggesting Mg partially entering the network in a different bioactive glass [41]. The latter paper suggested that in bioactive glasses Mg changes its structural role from network modifier to network former (*i.e.* acting as an intermediate oxide) depending on the Mg amount, resulting in formation of $[MgO_4]$ tetrahedra entering the silicate network in analogy to the role of aluminium ions in aluminosilicate glasses [41, 52, 53]. In silicate glasses, magnesium is typically present in four-, five- or even six-fold coordination [54–59]. MD simulation studies on Bioglass 45S5 revealed that magnesium is mainly present in five-fold coordination with a non-negligible amount of four- and small amounts of six-fold coordinated magnesium, depending on Mg content [51]. Despite the numerous studies on Mg-containing glasses, the role of Mg in the glass network is not yet fully understood. Nonetheless the amount of evidence pointing at magnesium playing a similar role as aluminium in silicate glasses is small, and most studies suggest that even in high network modifier content glasses, such as bioactive glasses, magnesium acts as a network modifier.

The influence of MgO for CaO substitution was not clear. When replacing one third of CaO with MgO, no effect on combined modifier (Ca + Mg) release was observed in dynamic ion release studies, but silicon concentrations decreased. When replacing two thirds of CaO with MgO, both modifier and silicon species release decreased. A decrease

in ion release with increasing MgO substitution can be explained by the more compact network (of a smaller molar volume) and the higher field strength of Mg^{2+} compared to Ca^{2+} . The non-continuous changes for modifier ion release observed here may also be caused by potential phase separation (fluoride-rich vs. silicate-rich) in the glass. Changes in ion release from these glasses can be expected to directly influence the setting behaviour of GIC: a slower ion release can be expected to result in more slowly setting cements and *vice versa*. These changes can also be exploited to tailor the handling properties (mixing plus setting) of GIC. This point will be discussed further below.

FTIR analyses after static dissolution experiments showed a change in glass structure during immersion in cell culture medium. The non-bridging oxygen band disappeared completely within 7 days owing to an ion exchange between modifier ions from the glass and protons from the Tris buffer solution and the subsequent formation of silanol (Si-OH) groups in the silicate network [60, 61]. Changes in spectra over time indicate formation of some phosphate surface layer, which is most pronounced for the Mg-free glass. Formation of a crystalline HAp layer was not detected within 7 days. This indicates only, however, that these glasses were slow in forming apatite in cell culture medium and that the time frame investigated here was simply too short. The immersion experiments here were performed in cell culture medium rather than in simulated body fluid. Cell culture medium contains various organic components, such as amino acids, which can have an effect on apatite mineralisation [62–64]. With increasing MgO for CaO substitution in the glass the phosphate band at about 1050 cm^{-1} (present at 7 days) became less pronounced. This may be an effect of the increased amount of Mg ions present, as Mg-containing glasses not only release ions more slowly than the corresponding Ca compositions (*cf.* above), but Mg ions are also known to impede apatite crystallisation [65, 66].

4.2 Glass ionomer cements

FTIR results suggest that with increasing MgO for CaO substitution in the glass, GIC set more slowly. These differences were most pronounced at the very early stages of cement setting, while all cements reached a constant $COO^-/COOH$ ratio at approximately the same time. This suggests that glasses with higher MgO substitution give more time for the initial mixing of the cements, *i.e.* they show a longer “*working time*”, while the time until the cements are fully set should be comparable. These results on GIC setting are in good agreement with the findings on ion

release from the glasses, where ions were released more slowly from Mg containing glasses.

Mechanical properties of GIC are strongly related to their microstructure [8, 67]. Reducing the glass particle size changed the cement microstructure and improved the compressive strength of the cements in the present study. On the one hand, a larger relative surface area of the glass particles increases the ion release from the glass, resulting in a larger number of released cations. As a result, more ionic bridges can be formed and the cement strength increases [68, 69]. On the other hand, incorporating an increasing number of smaller particles into the cement makes the cements more homogeneous and should therefore also improve mechanical performance.

Variations in glass composition also affected GIC mechanical properties. However, no continuous effect of MgO for CaO substitution on GIC compressive strength was found. Instead, the compressive strength was highest for the glass with intermediate MgO substitution (glass 15Mg). The initial increase in compressive strength for GIC formed using glass 15Mg compared to glass 0Mg can be explained by the higher field strength of Mg^{2+} ions compared to Ca^{2+} ions, resulting in stronger ionic cross-links and thus more stable cements. Further MgO substitution in the glass (from glass 15Mg to 30Mg), however, did not result in a further increase in compressive strength but instead in a decrease. This can be explained by the much lower ion release from glass 30Mg compared to glasses 15Mg and 0Mg. The total amount of metal cations available for cross-linking when forming GIC of glass 30Mg was therefore much lower than for the other two glasses, so that even the higher field strength of the Mg ions could not compensate for this. We therefore have two competing effects here, which both affect GIC mechanical properties: ion release during glass degradation and cement mixing and the effect of cation field strength on ionic cross-linking within the cement matrix.

No significant changes in compressive strength were observed with increasing storage time in deionised water. It is known that the setting reaction of dental GIC, i.e. the ionic cross-linking of polyalkenoate chains by metal cations released from the glass, continues for very long time periods [70], at least several months [71]. It is also known that this affects GIC mechanical properties, showing an improvement of mechanical properties with time, at least during the first weeks [71, 72]. By contrast, no increase in compressive strength with time was observed in the present study. Dental GIC, however, owe their mechanical properties to a very large extent to the cross-linking by high field strength aluminium cations. Removing aluminium ions from the glass significantly affects GIC me-

chanical performance, but also their stability in the presence of water. Even the mechanical properties of dental (i.e. aluminium-containing) GIC are affected by water, e.g. owing to ion release or water absorption [73–75] and this is likely to be more pronounced in aluminium-free GIC. It has also been stated previously that the bi-functionality of vinyl phosphonic acid impedes GIC maturation compared to GIC prepared with poly(acrylic acid) only, owing to sterical hindrance of cations [76].

Another factor affecting the mechanical properties of GIC is the storage medium used. In the present study, we investigated this effect by storing the cements either immersed in deionised water (direct contact to water), in an atmosphere of 100% relative humidity or within the PTFE mould only (with the only water present being that used for preparing the cement mixture). GIC compressive strength was highest for specimens stored inside the mould only (i.e. with no contact to external humidity), followed by GIC stored at 100% rH. Cements stored in water showed the lowest compressive strength. In other words, the less water the specimens were exposed to, the higher their compressive strength was. This indicates that compressive strength was affected by water absorption, an effect observed previously for Al-free GIC prepared using poly(acrylic acid) [17, 20].

To improve the compressive strength of GICs, an optimum between ion release from the glass and effectiveness of ions in ionic cross-linking of the polymer chains has to be found. The results for compressive strength obtained here are very low not only compared to dental (aluminium-containing) GIC but also compared to previous Al-free GIC using the same polymer [21]. It has to be noted, however, that the glass system here was not tailored for optimum GIC performance. Instead, it was designed to be simple, with a small number of components only, to allow for studying the influence of MgO for CaO substitution in the glass on both glass and GIC properties (“structure/property relationship”). We have shown previously that it is possible to obtain Al-free systems with much higher compressive strengths than the results shown here [21]. The study here allowed us, however, to improve our understanding of metal cation field strength on the setting of Al-free GIC, particularly the role of magnesium ions.

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References

- [1] S.M. Kenny, M. Buggy, Bone cements and fillers: A review, *Journal of Materials Science-Materials in Medicine*, 14, 2003, 923-938.
- [2] G. Lewis, Properties of acrylic bone cement: State of the art review, *J. Biomed. Mater. Res.*, 38, 1997, 155-182.
- [3] S.P. James, M. Jasty, J. Davies, H. Piehler, W.H. Harris, A fractographic investigation of PMMA bone-cement focusing on the relationship between porosity reduction and increased fatigue life, *J. Biomed. Mater. Res.*, 26, 1992, 651-662.
- [4] J.S. Wang, H. Franzen, S. Toksvig Larsen, L. Lidgren, Does vacuum mixing of bone-cement affect heat-generation - Analysis of 4 cement brands, *J. Appl. Biomater.*, 6, 1995, 105-108.
- [5] Y.K. Liu, J.B. Park, G.O. Njus, D. Stienstra, Particle-Impregnated bone-cement - An invitro study, *J. Biomed. Mater. Res.*, 21, 1987, 247-261.
- [6] T. Kindt Larsen, D.B. Smith, J.S. Jensen, Innovations in acrylic bone-cement and application equipment, *J. Appl. Biomater.*, 6, 1995, 75-83.
- [7] S.K. Sidhu, Glass-ionomer cement restorative materials: a sticky subject?, *Aust. Dent. J.*, 56, 2011, 23-30.
- [8] A. Moshaverinia, N. Roohpour, W.W.L. Chee, S.R. Schricker, A review of powder modifications in conventional glass-ionomer dental cements, *J. Mater. Chem.*, 21, 2011, 1319-1328.
- [9] A.D. Wilson, A hard decade's work: Steps in the invention of the glass-ionomer cement, *J. Dent. Res.*, 75, 1996, 1723-1727.
- [10] J.G. Joshi, Aluminium a neurotoxin which affects diverse metabolic reactions, *BioFactors*, 2, 1990, 163-9.
- [11] M.C. Blades, D.P. Moore, P.A. Revell, R. Hill, In vivo skeletal response and biomechanical assessment of two novel polyalkenoate cements following femoral implantation in the female New Zealand White rabbit, *Journal of Materials Science-Materials in Medicine*, 9, 1998, 701-706.
- [12] G. Cournot-Witmer, J. Zingraff, J.J. Plachot, F. Escaig, R. Lefevre, P. Boumati, et al., Aluminium localization in bone from hemodialyzed patients - Relationship to matrix mineralization, *Kidney Int.*, 20, 1981, 375-385.
- [13] B.F. Boyce, H.Y. Elder, H.L. Elliot, I. Fogelman, G.S. Fell, B.J. Junor, et al., Hypercalcemic Osteomalacia due to Aluminium toxicity, *Lancet*, 2, 1982, 1009-1013.
- [14] L. Bingel, D. Groh, N. Karpukhina, D.S. Brauer, Influence of dissolution medium pH on ion release and apatite formation of Bio-glass (R) 45S5, *Mater. Lett.*, 143, 2015, 279-282.
- [15] A. Hoppe, N.S. Güldal, A.R. Boccaccini, A review of the biological response to ionic dissolution products from bioactive glasses and glass-ceramics, *Biomaterials*, 32, 2011, 2757-2774.
- [16] D.S. Brauer, Bioactive Glasses—Structure and Properties, *Angew. Chem. Int. Ed.*, 2015, 2-24.
- [17] D.S. Brauer, E. Gentleman, D.F. Farrar, M.M. Stevens, R.G. Hill, Benefits and drawbacks of zinc in glass ionomer bone cements, *Biomedical Materials*, 6, 2011.
- [18] M. Darling, R. Hill, Novel polyalkenoate (glass-ionomer) dental cements based on zinc silicate-glasses, *Biomaterials*, 15, 1994, 299-306.
- [19] K. Hurrell-Gillingham, I.M. Reaney, I. Brook, P.V. Hatton, In vitro biocompatibility of a novel Fe₂O₃ based glass ionomer cement, *J. Dent.*, 34, 2006, 533-538.
- [20] D.S. Brauer, N. Karpukhina, G. Kedia, A. Bhat, R.V. Law, I. Radecka, et al., Bactericidal strontium-releasing injectable bone cements based on bioactive glasses, *Journal of the Royal Society Interface*, 10, 2013.
- [21] M. Fuchs, E. Gentleman, S. Shahid, R.G. Hill, D.S. Brauer, Therapeutic ion-releasing bioactive glass ionomer cements with improved mechanical strength and radiopacity, *Frontiers in Materials*, 2015, 11.
- [22] J.C. Elliot, Structure and chemistry of the apatites and other calcium orthophosphates, 1 ed., Elsevier, Amsterdam, New York, London, Tokyo 1994.
- [23] J. Aaseth, G. Boivin, O. Andersen, Osteoporosis and trace elements - An overview, *J. Trace Elem. Med Biol.*, 26, 2012, 149-152.
- [24] Y.C. Fredholm, N. Karpukhina, R.V. Law, R.G. Hill, Strontium containing bioactive glasses: Glass structure and physical properties, *J. Non-Cryst. Solids*, 356, 2010, 2546-2551.
- [25] S. Fagerlund, P. Ek, L. Hupa, M. Hupa, Dissolution Kinetics of a Bioactive Glass by Continuous Measurement, *J. Am. Ceram. Soc.*, 95, 2012, 3130-3137.
- [26] S. Fagerlund, L. Hupa, M. Hupa, Dissolution patterns of bio-compatible glasses in 2-amino-2-hydroxymethyl-propane-1,3-diol (Tris) buffer, *Acta Biomater.*, 9, 2013, 5400-5410.
- [27] S. Fagerlund, P. Ek, M. Hupa, L. Hupa, On determining chemical durability of glasses, *Glass Technology-European Journal of Glass Science and Technology Part A*, 51, 2010, 235-240.
- [28] M. Blochberger, L. Hupa, D.S. Brauer, Influence of zinc and magnesium substitution on ion release from 45S5 at physiological and acidic pH, *Biomedical glasses*, 1, 2015.
- [29] B. Dickey, R. Price, D. Boyd, Evidence of a complex species controlling the setting reaction of glass ionomer cements, *Dent. Mater.*, 32, 2016, 596-605.
- [30] E.M.D. Valliant, B.T.;Price, R.;Boyd, D.;Filiaggi, M.J., Fourier transform infrared spectroscopy as a tool to study the setting reaction in glass-ionomer cements, *Mater. Lett.*, 185, 2016, 256-259.
- [31] J.C. Leyte, L.H. Zuiderweg, H.J. Vledder, An i.r. investigation of polyion-counterion interactions, *Spectrochimica Acta Part A-Molecular Spectroscopy*, A 23, 1967, 1397-1407.
- [32] S. Crisp, M.A. Pringuer, Wardlewo.D, A.D. Wilson, Reactions in Glass Ionomer Cements: II. Infrared Spectroscopic Study, *J. Dent. Res.*, 53, 1974, 1414-1419.
- [33] L. Bardet, Cassanas-Fabre, G., Alain, M., Étude de la transition conformationnelle de l'acide polyacrylique syndiotactique en solution aqueuse par spectroscopie de vibration, *J. Mol. Struct.*, 24, 1975, 153-164.
- [34] J. Serra, P. Gonzalez, S. Liste, S. Chiussi, B. Leon, M. Perez-Amor, et al., Influence of the non-bridging oxygen groups on the bioactivity of silicate glasses, *Journal of Materials Science-Materials in Medicine*, 13, 2002, 1221-1225.
- [35] J.R. Jones, P. Sepulveda, L.L. Hench, Dose-dependent behavior of bioactive glass dissolution, *J. Biomed. Mater. Res.*, 58, 2001, 720-726.
- [36] W. Jastrzebski, M. Sitarz, M. Rokita, K. Bulat, Infrared spectroscopy of different phosphates structures, *Spectrochimica*

- Acta Part a-Molecular and Biomolecular Spectroscopy, 79, 2011, 722-727.
- [37] R.Z. LeGeros, O.R. Trautz, E. Klein, Two types of carbonate substitution in the apatite structure, *Experientia*, 25, 1969.
- [38] Y.A. Zheng, D.J. Huang, A.Q. Wang, Chitosan-g-poly(acrylic acid) hydrogel with crosslinked polymeric networks for Ni^{2+} recovery, *Anal. Chim. Acta*, 687, 2011, 193-200.
- [39] L.L. Hench, R.J. Splinter, W.C. Allen, Bonding mechanisms at the interface of ceramic prosthetic materials, *J. Biomed. Mater. Res.*, 5, 1971, 171-141.
- [40] H. Scholz, *Glas- Natur, Struktur und Eigenschaften*, Springer-Verlag Berlin Heidelberg New York London Paris Tokyo 1988.
- [41] S.J. Watts, R.G. Hill, M.D. O'Donnell, R.V. Law, Influence of magnesia on the structure and properties of bioactive glasses, *J. Non-Cryst. Solids*, 356, 2010, 517-524.
- [42] M.T. Souza, M.C. Crovace, C. Schroder, H. Eckert, O. Peitl, E.D. Zanotto, Effect of magnesium ion incorporation on the thermal stability, dissolution behavior and bioactivity in Bioglass-derived glasses, *J. Non-Cryst. Solids*, 382, 2013, 57-65.
- [43] A. Al-Noaman, S.C.F. Rawlinson, R.G. Hill, The role of MgO on thermal properties, structure and bioactivity of bioactive glass coating for dental implants, *J. Non-Cryst. Solids*, 358, 2012, 3019-3027.
- [44] J. Massera, L. Hupa, M. Hupa, Influence of the partial substitution of CaO with MgO on the thermal properties and in vitro reactivity of the bioactive glass S53P4, *J. Non-Cryst. Solids*, 358, 2012, 2701-2707.
- [45] D.S. Brauer, N. Karpukhina, R.V. Law, R.G. Hill, Structure of fluoride-containing bioactive glasses, *J. Mater. Chem.*, 19, 2009, 5629-5636.
- [46] W. Vogel, *Glass Chemistry*, 2nd ed., Springer, Berlin, Heidelberg, New York, London 1994.
- [47] D.E. Day, Mixed alkali glasses - Their properties and uses, *J. Non-Cryst. Solids*, 21, 1976, 343-372.
- [48] J. Kjeldsen, M.M. Smedskjaer, J.C. Mauro, R.E. Youngman, L.P. Huang, Y.Z. Yue, Mixed alkaline earth effect in sodium aluminosilicate glasses, *J. Non-Cryst. Solids*, 369, 2013, 61-68.
- [49] H. Morikawa, S. Lee, T. Kasuga, D.S. Brauer, Effects of magnesium for calcium substitution in P_2O_5 -CaO- TiO_2 glasses, *J. Non-Cryst. Solids*, 380, 2013, 53-59.
- [50] J. Massera, S. Fagerlund, L. Hupa, M. Hupa, Crystallization Mechanism of the Bioactive Glasses, 45S5 and S53P4, *J. Am. Ceram. Soc.*, 95, 2012, 607-613.
- [51] A. Pedone, G. Malavasi, M.C. Menziani, Computational Insight into the Effect of CaO/MgO Substitution on the Structural Properties of Phospho-Silicate Bioactive Glasses, *Journal of Physical Chemistry C*, 113, 2009, 15723-15730.
- [52] Y. Waseda, J.M. Toguri, The structure of molten binary silicate systems CaO-SiO₂ and MgO-SiO₂, *Metallurgical Transactions B-Process Metallurgy*, 8, 1977, 563-568.
- [53] Y. Tabira, Local structure around oxygen atoms in CaMgSi₂O₆ glass by O K-edge EXELFS, *Materials Science and Engineering B-Solid State Materials for Advanced Technology*, 41, 1996, 63-66.
- [54] M.C. Wilding, C.J. Benmore, J.A. Tangeman, S. Sampath, Evidence of different structures in magnesium silicate liquids: coordination changes in forsterite- to enstatite-composition glasses, *Chem. Geol.*, 213, 2004, 281-291.
- [55] P. Ildefonse, G. Calas, A.M. Flank, P. Lagarde, Low Z-elements (Mg, Al, and Si) K-edge X-ray-absorption spectroscopy in minerals and disordered-systems, *Nuclear Instruments & Methods in Physics Research Section B-Beam Interactions with Materials and Atoms*, 97, 1995, 172-175.
- [56] D. Li, M.S. Peng, T. Murata, Coordination and local structure of magnesium in silicate minerals and glasses: MgK-edge XANES study, *Can. Mineral.*, 37, 1999, 199-206.
- [57] L.A. Bugaev, A.P. Sokolenko, H.V. Dmitrienko, A.M. Flank, Fourier filtration of XANES as a source of quantitative information of interatomic distances and coordination numbers in crystalline minerals and amorphous compounds, *Physical Review B*, 65, 2002, 8.
- [58] C.D. Yin, M. Okuno, H. Morikawa, F. Marumo, Structure analysis of MgSiO₃ glass, *J. Non-Cryst. Solids*, 55, 1983, 131-141.
- [59] K. Shimoda, T. Nemoto, K. Saito, Local structure of magnesium in silicate glasses: A ²⁵Mg 3QMAS NMR study, *J. Phys. Chem. B*, 112, 2008, 6747-6752.
- [60] A.E. Clark, C.G. Pantano, L.L. Hench, Auger spectroscopic analysis of Bioglass corrosion films, *J. Am. Ceram. Soc.*, 59, 1976, 37-39.
- [61] L.L. Hench, Bioceramics - From Concept to Clinic, *J. Am. Ceram. Soc.*, 74, 1991, 1487-1510.
- [62] F.A. Shah, D.S. Brauer, N. Desai, R.G. Hill, K.A. Hing, Fluoride-containing bioactive glasses and Bioglass (R) 45S5 form apatite in low pH cell culture medium, *Mater. Lett.*, 119, 2014, 96-99.
- [63] F.A. Shah, D.S. Brauer, R.G. Hill, K.A. Hing, Apatite formation of bioactive glasses is enhanced by low additions of fluoride but delayed in the presence of serum proteins, *Mater. Lett.*, 153, 2015, 143-147.
- [64] F.A. Shah, D.S. Brauer, R.M. Wilson, R.G. Hill, K.A. Hing, Influence of cell culture medium composition on in vitro dissolution behavior of a fluoride-containing bioactive glass, *Journal of Biomedical Materials Research Part A*, 102, 2014, 647-654.
- [65] W. Kibalczyk, J. Christoffersen, M.R. Christoffersen, A. Zielenkiewicz, W. Zielenkiewicz, The Effect of Magnesium-Ions on the Precipitation of Calcium Phosphates, *J. Cryst. Growth*, 106, 1990, 355-366.
- [66] J. Ma, C.Z. Chen, D.G. Wang, Y. Jiao, J.Z. Shi, Effect of magnesia on the degradability and bioactivity of sol-gel derived SiO₂-CaO-MgO-P₂O₅ system glasses, *Colloids and Surfaces B-Biointerfaces*, 81, 2010, 87-95.
- [67] D. Xie, W.A. Brantley, B.M. Culbertson, G. Wang, Mechanical properties and microstructures of glass-ionomer cements, *Dent. Mater.*, 16, 2000, 129-138.
- [68] T. De Caluwe, C.W.J. Vercruysse, S. Fraeyman, R.M.H. Verbeeck, The influence of particle size and fluorine content of aluminosilicate glass on the glass ionomer cement properties, *Dent. Mater.*, 30, 2014, 1029-1038.
- [69] L.H. Prentice, M.J. Tyas, M.F. Burrow, The effect of particle size distribution on an experimental glass-ionomer cement, *Dent. Mater.*, 21, 2005, 505-510.
- [70] G.J. Mount, *Atlas of Glass Ionomer Cement*, 3rd ed., CRC Press, London, UK, 2003.
- [71] N. Zainuddin, N. Karpukhina, R.G. Hill, R.V. Law, A long-term study on the setting reaction of glass ionomer cements by Al-27 MAS-NMR spectroscopy, *Dent. Mater.*, 25, 2009, 290-295.
- [72] S.G. Griffin, R.G. Hill, Influence of glass composition on the properties of glass polyalkenoate cements. Part II: influence of phosphate content, *Biomaterials*, 21, 2000, 399-403.

- [73] B.E. Causton, The physico-mechanical consequences of exposing glass ionomer cements to water during setting, *Biomaterials*, 2, 1981, 112-115.
- [74] P. Mojon, R. Kaltio, D. Feduik, E.B. Hawbolt, M.I. MacEntee, Short-term contamination of luting cements by water and saliva, *Dent. Mater.*, 12, 1996, 83-87.
- [75] M.A. Cattaniolorente, C. Godin, J.M. Meyer, Mechanical-behavior of glass-ionomer cements affected by long-term storage in water, *Dent. Mater.*, 10, 1994, 37-44.
- [76] H.M. Anstice, J.W. Nicholson, Investigation of the post-hardening reaction in glass-ionomer cements on Poly(Vinyl Phosphonic acid), *Journal of Materials Science-Materials in Medicine*, 6, 1995, 420-425.