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ORIGINAL ARTICLE

Comparative evaluation of the calcium release from mineral trioxide aggregate and its mixture with glass ionomer cement in different proportions and time intervals – An *in vitro* study

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Abstract *Background:* Addition of glass ionomer cement (GIC) has been suggested to improve the setting time and handling characteristics of mineral trioxide aggregate (MTA). This study evaluated the effect of adding GIC to MTA in terms of calcium release, an issue that has not been previously studied.

Materials and methods: The study comprised four groups with five samples each: a control group of MTA alone and experimental groups I (1MTA:1GIC), II (2MTA:1GIC), and III (1MTA:2GIC) based on the mixture of MTA and GIC powders in the respective proportions by volume. Calcium release from the samples was measured by atomic absorption spectrophotometry at 15 min, 6 h, 24 h, and 1 week after setting. The level of statistical significance was set at $p < 0.05$.

Results: Groups I (1MTA:1GIC) and III (1MTA:2GIC) released significantly less calcium than the control group at all time periods, except at 15 min for group I. Group II (2MTA:1GIC) showed no significant difference in calcium release compared to the control at any time period. Group II exhibited greater calcium release than group I or III at all time periods, with significant differences between groups I and II at 1 week and between groups I and III at 24 h and 1 week. There were no statistical differences in calcium release between groups I and III.

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Conclusions: Adding GIC to improve the setting time and handling properties of the MTA powder can be detrimental to the calcium-releasing ability of the resultant mixture, depending on the proportion of GIC added. Adding MTA and GIC at a proportion of 2:1 by volume did not impact calcium release from the mixture. These findings should be verified through further clinical studies.

1. Introduction

Mineral trioxide aggregate (MTA) has many favorable biological properties for endodontic usage (Torabinejad and Parirokh, 2010). These properties are related to the formation of calcium hydroxide and subsequent release of calcium ions by MTA, as these actions modulate cytokine production and promote an alkaline pH, hydroxyapatite formation, and cementum deposition (Sarkar et al., 2005; Torabinejad et al., 1997; Zarrabi et al., 2010). However, despite these favorable properties, MTA has several drawbacks, including a prolonged setting time and difficult handling characteristics (Torabinejad et al., 1995). In this regard, the addition of accelerators and other vehicles to MTA has been recommended (Bortoluzzi et al., 2006; Gandolfi et al., 2008; Kogan et al., 2006; Wiltbank et al., 2007).

Combining glass ionomer cement (GIC) powder with MTA powder recently has been proposed as a means to overcome the drawbacks of MTA (Jeong et al., 2010; Oh et al., 2010). However, very few studies of this process have been conducted. One study evaluated the physical and chemical properties of experimental mixtures of MTA and GIC powders mixed at three different proportions. The experimental mixtures showed improved setting times but poor compressive strength and pH when compared to MTA powder alone (Jeong et al., 2010). Another study found that the biocompatibility of an experimental mixture of MTA and GIC was similar to the biocompatibility of MTA or GIC individually (Oh et al., 2010). Nevertheless, further studies have been recommended to determine the proper mixing ratio of GIC and MTA and the effect of different mixing ratios on various properties (Jeong et al., 2010).

The setting reaction of MTA results in the formation of calcium hydroxide, which subsequently dissociates into calcium and hydroxyl ions. The biological response to MTA is suggested to be based on its alkaline pH and calcium ion release (Camilleri and Pitt Ford, 2006; Holland et al., 1999, 2001; Massi et al., 2011; Yoshiba et al., 1996). Takita et al. (2006) confirmed that the proliferation of human dental pulp cells in contact with MTA is related to the continuous release of calcium ions. Therefore, any modification of MTA should not affect its calcium ion-releasing ability.

Because calcium ion release is the basis for the biological properties and applications of MTA, understanding the calcium ion-releasing abilities of GIC–MTA mixtures is of crucial clinical importance. However, no studies of this aspect have been conducted to date. Therefore, the purpose of this study was to compare calcium ion release among different GIC–MTA mixtures. The null hypothesis was that there would be no difference in calcium ion release from MTA–GIC mixtures containing different proportions of powders or measured at various times after setting.

2. Materials and methods

This study was designed to evaluate and compare the effect of combining GIC and MTA powders in three different proportions in terms of the calcium ion release from the mixture at different times after setting. The study included four groups with five samples each. Control group samples were prepared by mixing MTA powder (Angelus, Londrina, PR, Brazil) with distilled water. Samples in experimental groups I, II, and III were prepared by mixing MTA and GIC powders (Fuji II, GC Corporation, Tokyo, Japan) at proportions of 1:1, 2:1, and 1:2 by volume, respectively. Considering the powder/liquid ratio recommended by the manufacturer, the MTA and GIC powders were proportioned separately by using an automated weighing machine (Essae Teraoka Ltd., Singapore). Finally, powder mixtures in groups I, II, and III were mixed with liquid component of GIC, instead of distilled water provided with MTA, in order to fulfill the purpose of adding GIC to MTA.

Composition of MTA and GIC used in the study

MTA	GIC
Powder – silica, potassium oxide, alumina, sodium oxide, iron oxide, sulfur trioxide, calcium oxide, bismuth oxide, magnesium oxide and insoluble residues of calcium oxide, potassium sulfate, sodium sulfate and crystalline silica	*Powder* – silica, alumina, aluminum fluoride, calcium fluoride, sodium fluoride, aluminum phosphate
Liquid – distilled water	*Liquid* – tartaric acid, co-polymers of itaconic, maleic or tricarboxylic acid

Study samples were prepared in a Teflon mold. After setting, samples were placed in polypropylene tubes containing 10 ml of distilled water that had been confirmed to be free of calcium ions and to have a neutral pH of 6.8. Tubes containing the samples were closed and maintained at room temperature during the study.

Calcium ion release from the samples was measured at 15 min, 6 h, 24 h, and 1 week after sample setting by using an atomic absorption spectrometer (Varian, Model No. AA240) that was equipped with a specific cathode lamp for reading. The instrument was calibrated and used in accordance with the manufacturer's instructions. The amount of calcium ions that were released was measured as follows. First, the sample was removed, and the distilled water from the tube was emptied into the spectrometer flask. Lanthanum solution was added to prevent possible interference by other alkaline metals. The amount of released calcium ions (in parts per million, ppm) was measured. Finally, the distilled water was

discarded, fresh distilled water was added, and the samples were placed in tubes until the next time for evaluation.

The results were statistically analyzed by using the SPSS 20 software package (Statistical Package for the Social Sciences 20). Statistical analysis was done by using a one-way analysis of variance (ANOVA) and Tukey's post hoc test for multiple comparisons.

3. Results

Mean amounts of released calcium ions in the present study are shown in Fig. 1 and Table 1. The control group (only MTA) showed the highest amount of calcium release among all groups at all time periods. Compared to the control group, groups I (1MTA:1GIC) and III (1MTA:2GIC) released significantly less calcium at all time periods except at 15 min for group I. Group II (2MTA:1GIC) showed no significant difference in calcium release compared to the control group at any time period. Calcium release from group II was greater than calcium release from group I or III at all time periods, with statistically significant differences between groups I and II at 1 week and between groups II and III at 24 h and 1 week. No statistically significant differences were found between groups I and III.

4. Discussion

In the present study, GIC was combined with MTA to modify the setting time and handling characteristics of MTA. Calcium ion release from all the groups was time dependent. Furthermore, greater calcium ion release was observed in the control group compared to the experimental groups. Therefore, the null hypothesis of the study was rejected.

The GIC and MTA powders were proportioned in accordance with a previously described methodology (Jeong et al., 2010). Calcium ion release into the solution has been demonstrated to increase to a maximum at 7 days, with a decline thereafter (Duarte et al., 2000). Therefore, calcium ion release in this study was measured up to 1 week after setting. The use of atomic absorption spectrophotometry to measure calcium ion release is well established. This analytical technique measures the absorption of light by elements to measure their concentrations. This method delivers rapid and accurate results, provides elemental selectivity, and is easy to use (Duarte et al., 2000; Tanomaru-Filho et al., 2009).

Calcium ion release from all the groups typically increased with time, although the experimental groups released fewer calcium ions than the control group. These results suggest that the addition of GIC affected the calcium ion-releasing ability of MTA. Experimental groups based on the 1MTA:1GIC and 1MTA:2GIC mixtures showed significantly less calcium ion release compared to the control group without GIC. This result suggests that the decline in calcium release depended on the proportion of GIC that had been added relative to MTA. GIC added at a 1:1 or 2:1 ratio to MTA had a greater effect on calcium release than GIC added at a 1:2 ratio. This significant effect was noted at all time periods, although adding GIC and MTA at equal proportions did not significantly affect calcium release 15 min after setting.

The reduced calcium release from groups containing MTA-GIC mixtures may be attributed to the setting reaction of GIC, although no previous studies have been conducted on this subject. The setting reaction of GIC is well known (Bayne and Thompson, 2005; Mount, 1988; Shen, 2003; Wilson and

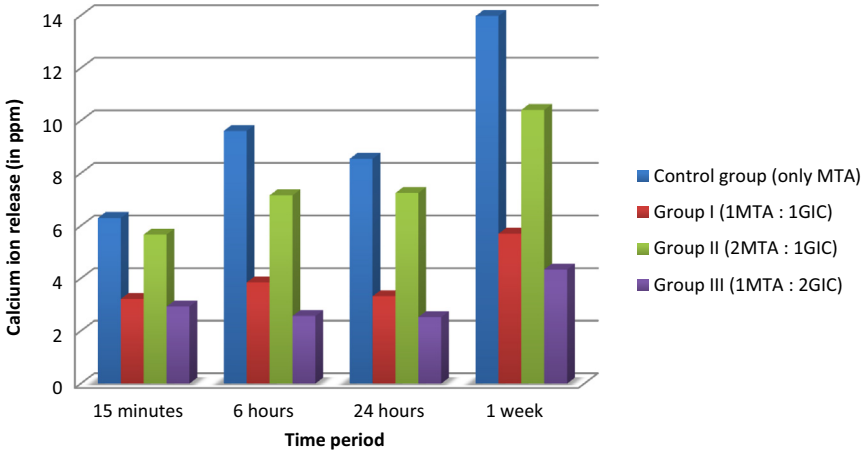

Figure 1 Mean calcium ion release (in ppm) from the study groups at different time periods.

Table 1 Statistical analysis of the mean calcium ion release (in ppm) from the study groups at different time periods.

	15 min Mean ± SD	6 h Mean ± SD	24 h Mean ± SD	1 week Mean ± SD
Control group (only MTA)	6.30^{a} ± 1.38	9.61^{a} ± 0.64	8.55^{a} ± 2.55	13.98^{a} ± 3.07
Group I (1MTA:1GIC)	3.22^{ab} ± 2.49	3.85^{b} ± 2.74	3.32^{bc} ± 3.03	5.70^{b} ± 3.33
Group II (2MTA:1GIC)	5.67^{ab} ± 2.10	7.16^{ab} ± 1.93	7.25^{ab} ± 2.06	10.41^{a} ± 2.61
Group III (1MTA:2GIC)	2.93^{b} ± 1.69	2.57^{b} ± 1.42	2.53^{c} ± 1.91	4.34^{b} ± 1.94
F	9.223	25.687	12.836	23.466
p value	< 0.001	< 0.001	< 0.001	< 0.001

Different superscript letters indicate a significant difference between groups.

McLean, 1988) and involves the dissolution of peripheral portions of the silicate glass particles by polyacrylic acid-based liquid, leading to the release of calcium, aluminum, and other ions. Calcium ions chelate carboxyl groups on polyacrylic acid, producing an amorphous polymer gel (Bayne and Thompson, 2005). During the next 24–72 h, the calcium ions are replaced by more slowly reacting aluminum ions to produce a highly cross-linked matrix (Bayne and Thompson, 2005).

In the experimental groups, the GIC–MTA powder was mixed with GIC liquid that, while containing water, would preferentially react with the GIC powder over the MTA powder. Consequently, the GIC sets prematurely relative to the MTA, leading to a rigid matrix or sheath that can entrap released calcium ions. Furthermore, because the initial setting reaction of GIC is dependent on calcium ions, there is a possibility that the MTA-released calcium ions will be consumed in the setting reaction of GIC (Mount, 1988; Shen, 2003; Wilson and McLean, 1988). Although the precise interaction between GIC liquid and MTA is still unknown, the above mechanisms may hypothetically explain the reduced calcium ion release in the experimental groups.

Unlike the 1MTA:1GIC and 1MTA:2GIC mixtures, the 2MTA:1GIC mixture showed no significant decline in calcium release compared to the control group. Therefore, it can be deduced that lower proportions of GIC compared to MTA in the mixtures lead to less-detrimental effects on the calcium ion release. Additionally, the 2MTA:1GIC mixture released significantly more calcium than either the 1MTA:1GIC or 1MTA:2GIC mixture, except at 15 min. This finding suggests that the presence of GIC at a 1:2 proportion relative to MTA may not be as detrimental to the calcium release as its presence at a 1:1 or 2:1 proportion. In terms of improving the setting time and handling characteristics of MTA, only adding GIC to MTA in a 1:2 proportion can be considered to be clinically relevant. However, this conclusion requires further analysis, particularly of the interaction between MTA and GIC powder and liquid, before any clinical recommendations can be made.

5. Conclusion

Combining GIC and MTA powders to improve the setting time and handling properties of MTA can be detrimental to the calcium-releasing ability of the resulting mixture, depending on the proportion of GIC added relative to MTA. However, GIC added at a 1:2 proportion relative to MTA showed no significant decline in calcium release and can be recommended for the above purpose. Nevertheless, this conclusion requires further clinical validation.

Ethical statement

This study does not require ethical approval.

Conflict of interest

The author has no financial interest associated with the aforementioned materials used in this study and declares no conflict of interest.

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