
The chemical constitution and biocompatibility of accelerated Portland cement for endodontic use

J. Camilleri^{1,2}, F. E. Montesi², L. Di Silvio¹ & T. R. Pitt Ford¹

¹Department of Conservative Dentistry, and Biomaterials Research of the Guy's, King's and St Thomas' Dental Institute, King's College London, UK; and ²Department of Building and Civil Engineering, Faculty of Architecture and Civil Engineering, University of Malta, Malta

Abstract

Camilleri J, Montesi FE, Di Silvio L, Pitt Ford TR. The chemical constitution and biocompatibility of accelerated Portland cement for endodontic use. *International Endodontic Journal*, **38**, 834–842, 2005.

Aim To evaluate the biocompatibility of mineral trioxide aggregate and accelerated Portland cement and their eluants by assessing cell metabolic function and proliferation.

Methodology The chemical constitution of grey and white Portland cement, grey and white mineral trioxide aggregate (MTA) and accelerated Portland cement produced by excluding gypsum from the manufacturing process (Aalborg White) was determined using both energy dispersive analysis with X-ray and X-ray diffraction analysis. Biocompatibility of the materials was assessed using a direct test method where cell proliferation was measured quantitatively using Alamar Blue™ dye and an indirect test method where cells were grown on material elutions and cell proliferation was assessed using methyltetrazolium assay as recommended by the International standard guidelines, ISO 10993-Part 5 for *in vitro* testing.

Results The chemical constitution of all the materials tested was similar. Indirect studies of the eluants showed an increase in cell activity after 24 h compared with the control in culture medium ($P < 0.05$). Direct cell contact with the cements resulted in a fall in cell viability for all time points studied ($P < 0.001$).

Conclusions Biocompatibility testing of the cement eluants showed the presence of no toxic leachables from the grey or white MTA, and that the addition of bismuth oxide to the accelerated Portland cement did not interfere with biocompatibility. The new accelerated Portland cement showed similar results. Cell growth was poor when seeded in direct contact with the test cements. However, the elution made up of calcium hydroxide produced during the hydration reaction was shown to induce cell proliferation.

Keywords: biocompatibility, chemical constitution, mineral trioxide aggregate, prototype cements.

Received 28 April 2005; accepted 20 July 2005

Introduction

Mineral trioxide aggregate (MTA) is used mostly in endodontics. Until recently two commercial forms of MTA have been available (ProRoot MTA, Tulsa Dental Products, Tulsa, OK, USA); namely the grey and the

white MTA (GMTA and WMTA) both with similar chemical and physical properties. Recently MTA-Angelus (Angelus Soluções Odontológicas, Londrina, Brazil) has also become available on the market. MTA is essentially Portland cement with 4 : 1 proportions of bismuth oxide added for radiopacity (Torabinejad & White 1995). Camilleri *et al.* (2005a) have shown that MTA is composed primarily of tricalcium and dicalcium silicate, which on hydration produce a silicate hydrate gel and calcium hydroxide, thus rendering it biocompatible. Materials with similar chemical composition

Correspondence: Dr Josette Camilleri, Department of Building and Civil Engineering, Faculty of Architecture and Civil Engineering, University of Malta, Malta (Tel.: +356 99485747; e-mail: joz@global.net.mt).

but which do not produce calcium hydroxide were shown to be less biocompatible (Camilleri *et al.* 2005b).

One of the main disadvantages with using MTA is its extended setting time and difficult handling. In industry the setting time of Portland cement is controlled by grinding the cement with gypsum at the end stage of the manufacturing process. The gypsum is added by cement manufacturers to retard the setting time of the cement clinker. Removal of gypsum results in a flash-set which can be controlled by the use of a superplasticising admixture which makes the material more workable. Significant reduction in setting time could be advantageous when using the material as the number of visits could be reduced.

There is conflicting data on the *in vivo* biocompatibility of GMTA and WMTA. Holland *et al.* (1999, 2001, 2002) showed that both types were biocompatible when implanted in rat connective tissue; however the materials were not tested in the same experiment. In contrast, Perez *et al.* (2003) showed that WMTA was not as biocompatible as the grey version and postulated that the difference might be due to surface morphology of the materials. Camilleri *et al.* (2004) showed no difference between the two variants, however both materials exhibited reduced cell growth when cured for 28 days. Thus, aged material may not be as biocompatible as freshly mixed material. This is another indication that biocompatibility could be related to the amount of calcium hydroxide produced during the hydration reaction.

The biocompatibility of MTA has been previously investigated *in vitro* using a number of the cell lines; MG 63 cells (Koh *et al.* 1997, 1998, Mitchell *et al.* 1999), Saos2 cells (Camilleri *et al.* 2004, 2005b), human osteoblasts (Zhu *et al.* 2000, Perez *et al.* 2003), and L929 mouse fibroblasts (Haglund *et al.* 2003). However since calcium hydroxide is produced as a by-product of the hydration reaction, it is imperative to ensure that

there is no reaction between the material and the reagents used in the experimental procedure. Koh *et al.* (1997, 1998) and Abdullah *et al.* (2002) used 2.5% glutaraldehyde in phosphate buffer to fix the cells prior to scanning electron microscopy. The phosphate in the fixing buffer reacts with the calcium hydroxide producing calcium phosphate crystals that could be identified under the scanning electron microscope (SEM) (Camilleri *et al.* 2005a). Critical point drying which is an essential step to dry the fixed specimens prior to microscopy causes carbonation of the cement surfaces (Camilleri *et al.* 2004). As a result of these findings, an optimised method was developed to evaluate biocompatibility in order to ensure that there is no interference from the medium with the by-products of the setting reaction. Cell metabolic function was assessed using indicators that test for specific enzymes present within living cells. Cell proliferation was measured with a redox indicator as proliferating cells maintain a reducing environment. The aim of this study was to evaluate the biocompatibility of MTA and related accelerated materials using a new and modified technique.

Materials and methods

The materials used in this study are shown in Table 1. Proto B was produced by adding bismuth oxide to the cement in 1 : 4 proportions (Sigma, Aldrich, Dorset, UK) thus having a material with a chemical constitution similar to that of MTA. Grey Portland cement (GOPC; Italcementi SPA, Bergamo, Italy), white Portland cement (WOPC; Lafarge Asland, Valencia, Spain) and both variants of MTA were mixed with water while both prototype cements were mixed with water and superplasticizer (Degussa Construction Chemicals, Manchester, UK). The super-plasticiser was used to obtain a homogeneous flowable mix as the lack of gypsum tended to flash-set the cement.

Table 1. Raw materials used for the biocompatibility study

Abbreviation	Material	Manufacturing company
GOPC	Grey Portland cement	Italcementi SPA, Bergamo, Italy
WOPC	White Portland cement	Lafarge Asland, Valencia, Spain
GMTA	Grey mineral trioxide aggregate	ProRoot MTA, Tulsa Dental Products, Tulsa, OK, USA
WMTA	White mineral trioxide aggregate	ProRoot MTA, Tulsa Dental Products, Tulsa, OK, USA
Proto A	White Portland cement clinker inter-ground without the gypsum	Aalborg White, Aalborg, Denmark
Proto B	White Portland cement clinker inter-ground without the gypsum and mixed with 4 : 1 proportion bismuth oxide	Aalborg White, Aalborg, Denmark Sigma –Aldrich, Dorset, UK

Chemical constitution and particle size measurement

Energy Dispersive Analysis by X-ray (EDAX) was performed under the SEM (Leo 1430; Philips, Cambridge, UK). A thin layer of powder was dispersed over carbon double-sided tape attached to an aluminium stub (Agar Scientific, Stansted, UK). The stubs were carbon coated (Agar Scientific) for electrical conductivity. The specimens were then viewed under the SEM and X-ray analysis was carried out to determine the constituent elements of the powders. Two stubs were made for each material and the analysis was performed twice for each sample. In addition, phase analysis was carried out using X-ray diffraction (Ital Structures Compact 3K5, Riva del Garda, Italy). The diffractometer used Cu K α radiation at 30 mA and 40 kV. The crystalline structure of the test cement was determined by passing a beam of X-rays of known wavelength into the specimen while rotating it through an angle θ . The intensity of X-rays from the sample was measured by a detector. The detector was rotated between 10–80° at 0.02° θ per 0.5 s. Phase identification was accomplished by use of search-match software utilizing International Centre for Diffraction Data (ICDD) database. Electron micrographs were produced in order to measure particle sizes of the cements.

Biocompatibility

The biocompatibility of GOPC and WOPC, white Portland cement clinker inter-ground without the gypsum (Proto A) and Proto B (Aalborg White, Aalborg, Denmark) and both variants of MTA was assessed using a human osteosarcoma cell line (HOS TE 85 ECACC No.87070202).

Cell culture

Cells were cultured at 37 °C in a humidified atmosphere with 5% CO₂ in Dulbecco's modified eagles medium (DMEM), supplemented with 10% foetal calf serum (FCS), 0.02 mol L⁻¹ HEPES, [4-(2-hydroxyethyl)-1-piperazineethanesulphonic acid], 2 mmol L⁻¹ L-Glutamine, 1% penicillin/streptomycin (Life Technologies, Invitrogen Ltd, Paisley, UK) and 150 μ g mL⁻¹ Ascorbate (Sigma, Aldrich, Dorset, UK).

Material preparation

The Portland cements were mixed at a water : cement ratio of 0.30 and prototype cements at 0.27. One sachet of MTA was mixed with the liquid provided and

pre-dosed by the manufacturer (Tulsa Dental Products). The cements were cast to produce discs 10 mm in diameter. Two discs of test material were used for the indirect test and three discs were used for the direct study.

Biocompatibility study

The biocompatibility of the test materials was evaluated *in vitro* according to ISO 10993-Part 5 (1992) using an indirect and direct testing method. In the indirect test the cytotoxicity of the eluant was evaluated. Two replicates per material were tested by placing 12 mm discs in 10 mL of DMEM (without FCS, to prevent any bacterial growth). A volume of 3 mL was removed from each test sample and replaced with fresh medium at each time interval. The test samples were maintained on a roller mixer (Luckham 4RT, Burgess Hill, UK) for the duration of the elution period.

The methyltetrazolium (MTT) assay (Mosmann 1983) was used to assess cell metabolic function. Seeding with HOS cells (1×10^4 cells mL⁻¹) of 96-well plates was performed and these were incubated for 24 h. The medium was then removed and replaced with 100 μ L of test eluants, which had previously been restored to 10% FCS. Eight replicates per elution were used thus giving a total of 16 wells tested per material under study. Elution medium from 1, 3, 7, 14 and 28 days was assessed at 24, 48 and 72-h cell exposure time. Standard culture medium was used as a negative nontoxic control. The MTT assay was performed for each time point.

For the direct biocompatibility test the Alamar Blue™ (Serotec, Kidlington, Oxford, UK) was used. Three replicates were used for each time point. The materials were cast as in the previous experiment and were cured at 37 °C and 100% humidity for 7 days. After 7 days of curing, the samples were washed with absolute alcohol for 3 min, air-dried and transferred to a sterile 24-well plate. Thermanox™ (NUNC brand products, Rochester, USA) (TMX) was used as the negative nontoxic control. The cells were seeded directly on the test materials and controls at 3.6×10^5 cells mL⁻¹ and incubated for 24 h at 37 °C in a humidified atmosphere of 5% CO₂ and 95% air. At selected time points 3, 7, 14 and 21 days, medium was removed from the wells containing the test disks. An aliquot of 1 mL of Alamar Blue™ diluted 1 : 10 in phenol red-free medium was added to each well and incubated for a further 4 h at 37 °C, 5% CO₂. Wells without any cells were used as the blank control. Following the incubation 8 \times 100 μ L aliquots from each well were taken and transferred to a 96-well plate for reading. Absorbance was read on a fluorescent

plate reader on emission wavelength of 590 nm (excitation wavelength 560 nm). The early age (cured for 1 day) behaviour of the cements was also evaluated by this method at 1, 3, 5, and 7 days. Statistical analysis was performed using the Pairwise *t*-test with $P = 0.05$.

Results

Chemical constitution

The EDAX of the powders is shown in Fig. 1, and X-ray diffraction analysis (XRD) is shown in Fig. 2. The EDAX

showed that the materials were composed of similar elements namely calcium, silicon and aluminium. The white materials differed from the grey by an absence of iron, and the MTA from the Portland cement by the presence of bismuth. Proto A and B had a similar elemental composition to the other cements; these two materials lacked iron and sulphur and Proto B contained bismuth. The XRD of the materials showed that they all were composed primarily of tricalcium silicate (ICDD 86-0402), and to a lesser amount dicalcium silicate (ICDD 1-1012). Both variants of MTA and Proto B showed the presence of bismuth oxide (ICDD 41-1449).

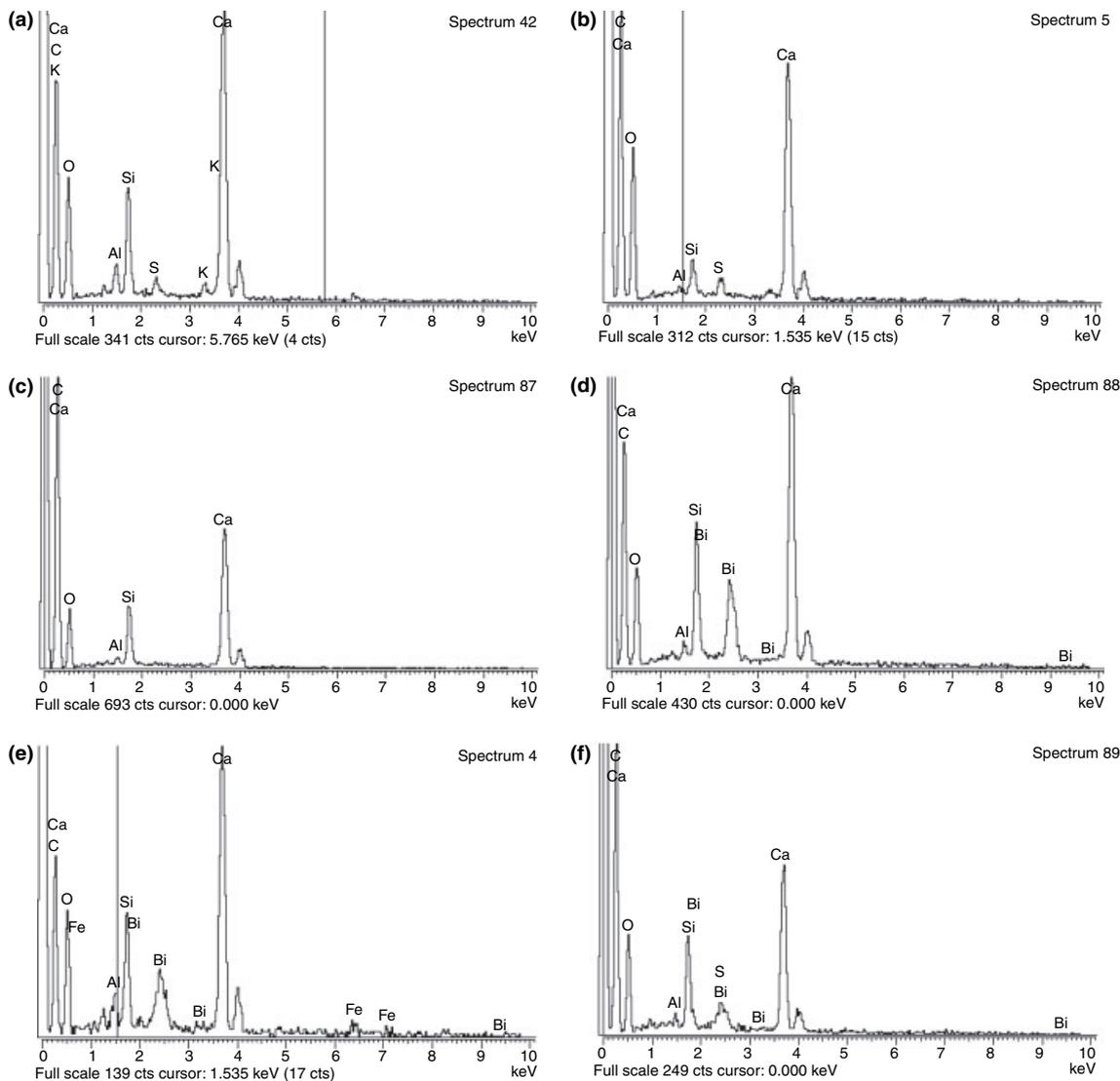


Figure 1 Energy dispersive analysis with X-ray of cements; (a) grey Portland cement; (b) white Portland cement (c) Proto A; (d) Proto B; (e) grey mineral trioxide aggregate; (f) white mineral trioxide aggregate showing the elemental peaks of each material.

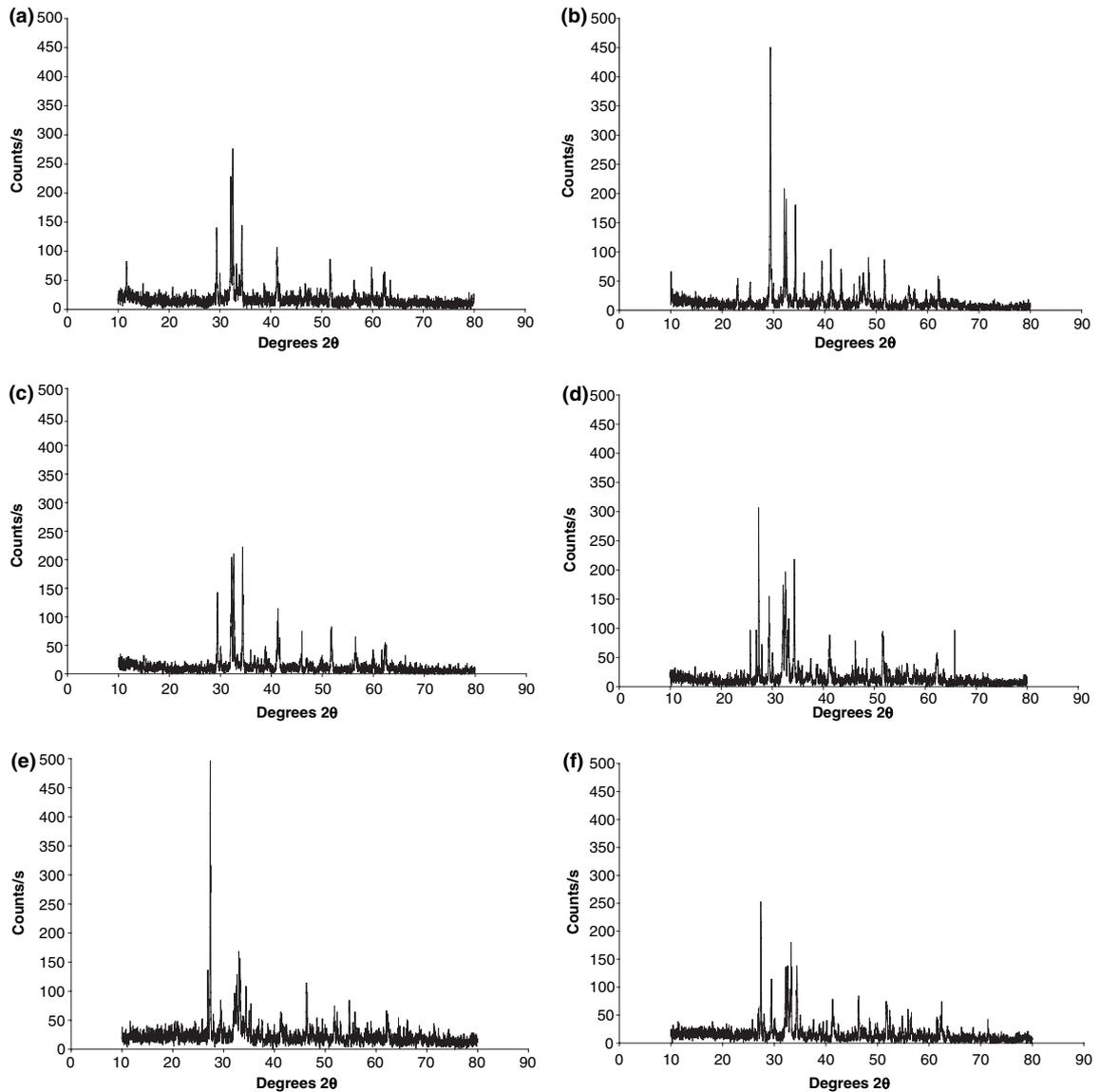


Figure 2 X-ray diffraction patterns of cements (a) grey Portland cement; (b) white Portland cement; (c) Proto A; (d) Proto B; (e) grey mineral trioxide aggregate; (f) white mineral trioxide aggregate showing the main phases of each material namely tricalcium and dicalcium silicate present in all materials tested.

Particle size measurement from scanning electron micrographs showed GOPC to be the most coarse having a mean particle size of 10–80 μm . WOPC and GMTA had a similar particle size distribution (range 5–40 μm). Proto A and Proto B had smaller particle size distribution (range 3–30 μm). WMTA had the smallest particles (range 3–20 μm). Another observation was the different particle shape of the bismuth oxide in MTA from that in Proto B; the bismuth oxide particles in MTA were elongated in contrast to those in Proto B that were round.

Biocompatibility

Generally all the results from the MTT assay excluded any severe deleterious effects of any leachables from the cements, with metabolic activity of cells detected at 24, 48 and 72 h (Fig. 3a–e). Cells exposed to eluants from commercial GMTA showed a drop in metabolic activity at 1 day ($P < 0.001$) and at 7 days ($P < 0.001$) following 48-h cell exposure, with an improvement at 72 h (Fig. 3a,c). However, both GMTA and Proto B

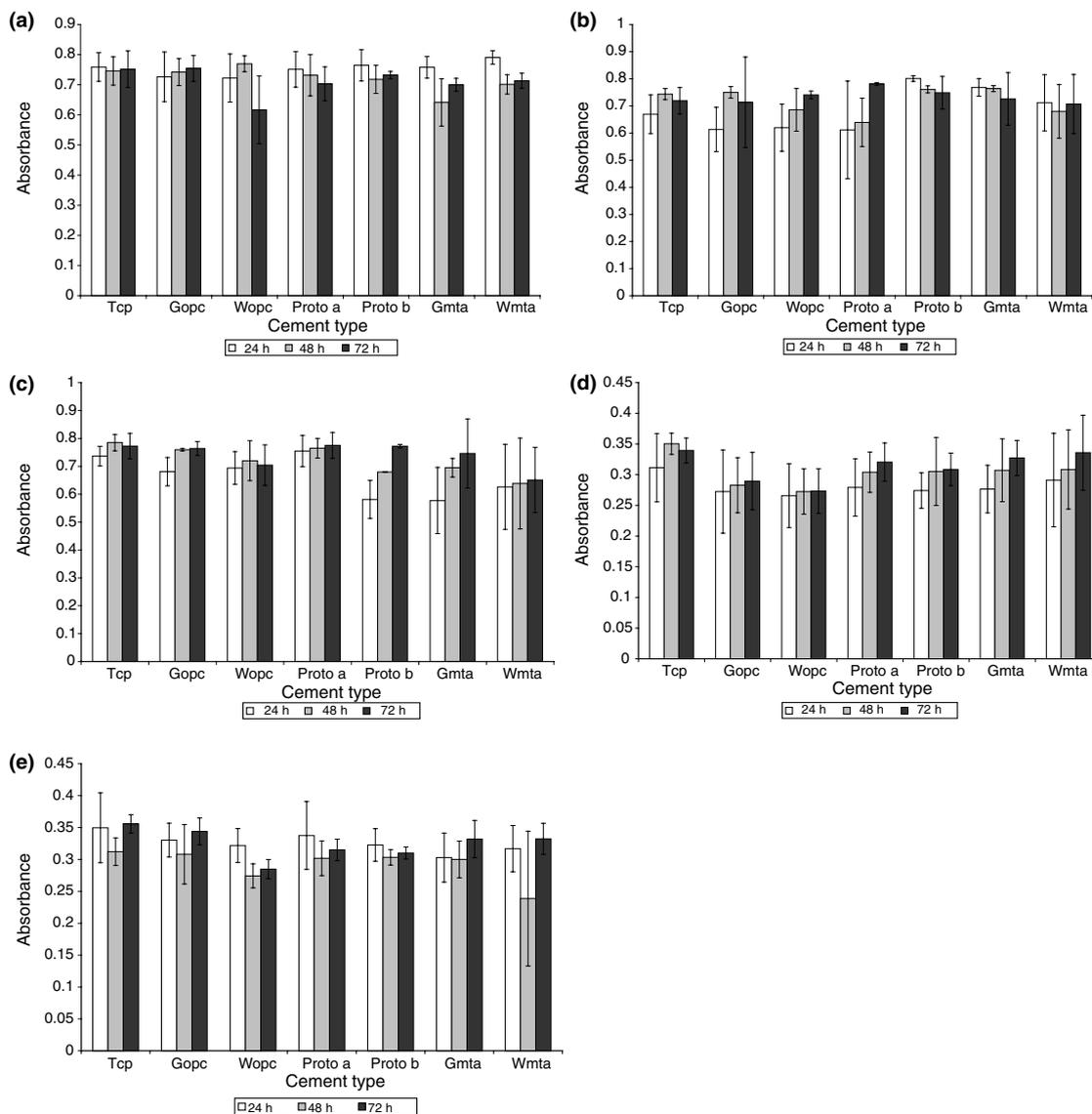


Figure 3 (a) Materials biocompatibility: mean absorbance values in methyltetrazolium (MTT) assay 1-day elution for the different cement types using an indirect test method. Cells were exposed for a period of 24, 48 and 72 h. Tissue culture plastic (tcp) was used as the negative control (i.e. nontoxic control). Results are \pm SD showing level of absorbance of blue formazan produced by viable cells ($n = 16$). (b) Materials biocompatibility: mean absorbance values in MTT assay of 3-day elution for the different cement types using an indirect test method. Cells were exposed for a period of 24, 48 and 72 h. Tissue culture plastic (tcp) was used as the negative control (i.e. nontoxic control). Results are \pm SD showing level of absorbance of blue formazan produced by viable cells ($n = 16$). (c) Materials biocompatibility: mean absorbance values in MTT assay of 7-day elution for the different cement types using an indirect test method. Cells were exposed for a period of 24, 48 and 72 h. Tissue culture plastic (tcp) was used as the negative control (i.e. nontoxic control). Results are \pm SD showing level of absorbance of blue formazan produced by viable cells ($n = 16$). (d) Materials biocompatibility: mean absorbance values in MTT assay of 14-day elution for the different cement types using an indirect test method. Cells were exposed for a period of 24, 48 and 72 h. Tissue culture plastic (tcp) was used as the negative control (i.e. nontoxic control) results are \pm SD showing level of absorbance of blue formazan produced by viable cells ($n = 16$). (e) Materials biocompatibility: mean absorbance values in MTT assay of 28-day elution for the different cement types using an indirect test method. Cells were exposed for a period of 24, 48 and 72 h, tcp was used as the negative control (i.e. nontoxic control). Results are \pm SD showing level of absorbance of blue formazan produced by viable cells ($n = 16$).

showed an enhanced cell activity in the 3-day elution fluid compared with the control medium (Fig. 3b). After 24-h exposure all test eluants showed an increase in cell metabolic activity ($P < 0.05$ pairwise *t*-test) with the exception of GMTA ($P < 0.001$) and proto B ($P < 0.001$) which were less biocompatible at 7-day elution after 24-h cell exposure. Results after 48 and 72-h cell exposure showed slight variations in cell behaviour. Proto B showed variable cell activity after 48-h cell exposure in the 7-day elution ($P = 0$, Fig. 3c). A fall in cell activity was observed at 14 and 28 days ($P = 0$, Fig. 3d,e) for the WOPC, and 14 days ($P = 0$, Fig. 3d) for the GOPC compared with the control.

The results for the proliferation (direct) study on materials cured for 7 days compared with TMX (tissue culture control surface) are shown in Fig. 4a. An increase in cell proliferation was observed for the time periods studied on the TMX. GMTA showed an enhancement in cell proliferation at 1 and 3 days of curing ($P < 0.05$). All the test cements did not encourage cell proliferation at all time intervals ($P < 0.001$). Results for direct contact of cells with materials cured for 1 day are shown in Fig. 4b. All the test materials showed a similar trend in proliferation, however, this was significantly lower than the TMX control ($P < 0.001$).

Discussion

All the materials tested in this study had a similar chemical constitution as verified by both EDAX and

XRD, and were composed primarily of tricalcium and dicalcium silicate. Both variants of MTA and Proto B had bismuth oxide added and the grey cements had traces of iron. Proto A and B differed from the rest of the cements by lacking the sulphur peak, which can be inferred as an absence of gypsum. Thus, on the addition of water, calcium silicate hydrate gel and calcium hydroxide would be produced.

The WOPC, Proto A and WMTA were finer than the GOPC and GMTA. WOPC has a lower alkali content because of volatilization during manufacture and the chemical composition of the raw materials (Lea 2001). This causes less ettringite formation during hydration. Ettringite is a complex hydrate made up of calcium, aluminium, silicon and sulphur, which deposits as needle-like crystals. The lower alkali content increases the solubility of calcium oxide and decreases the solubility of the sulphate ion. Thus, less sulphate is available to react with the tricalcium aluminate. The consequence is a reduction in strength. This is controlled by inter-grinding the clinker to finer particles to enhance its performance (Lea 2001). Particle measurement from scanning electron micrographs showed GMTA to have a similar particle size to WOPC. GMTA must have been derived from rapid hardening Portland cement, which is ground down to smaller sized particles to increase the rate of reaction and the early age strength. Increase in cement fineness necessitates the use of a higher water/cement ratio to be able to wet all the particles. WMTA was the finest of all the cements tested. This would explain the different handling of the

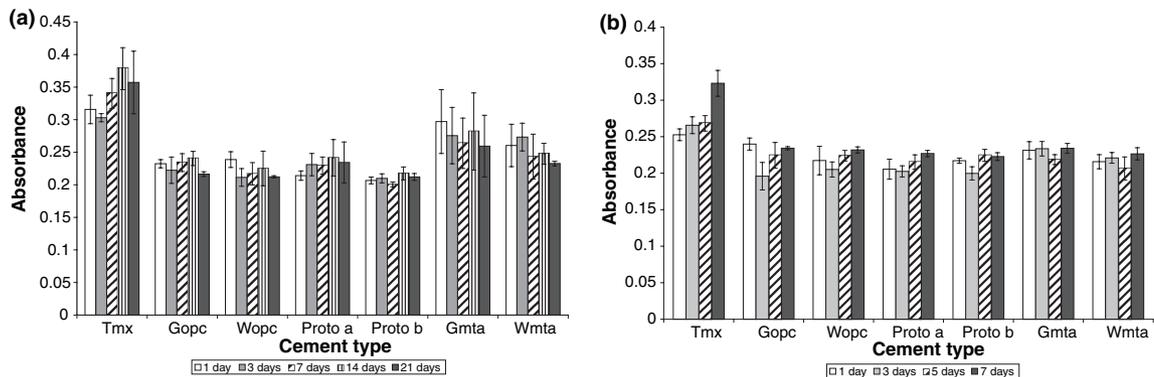


Figure 4 (a) Materials biocompatibility: mean absorbance values in Alamar Blue™ direct proliferation assay for 7-day cured materials. Proliferation was assessed over a period of 21 days the negative nontoxic control was Thermanox™ results are \pm SD showing level of absorbance of Alamar Blue™ for each cement ($n = 24$). (b) Materials biocompatibility: mean absorbance values in Alamar Blue™ direct proliferation assay for 1-day cured materials. Proliferation was assessed over a period of 7 days the negative nontoxic control was Thermanox™. Results are \pm SD showing level of absorbance of Alamar Blue™ for each cement ($n = 24$).

material at the similar water/cement ratios used to mix the grey variant. This problem did not arise with the prototype materials as a superplasticising admixture was added to the mixing water. The use of the admixture reduces the water required by the mix to achieve the same workability.

The biocompatibility of Portland cement has previously been documented (Abdullah *et al.* 2002). Holland *et al.* (1999, 2001, 2002) have shown similar mechanisms of action of Portland cement, MTA and calcium hydroxide. Saidon *et al.* (2003) claimed that both MTA and Portland cement have the same physical, chemical and biological properties. Camilleri *et al.* (2005a) have demonstrated that both Portland cement and MTA are composed of tricalcium and dicalcium silicate which on hydration produce calcium silicate hydrate gel and calcium hydroxide. In the present study the biocompatibility of the cements was determined quantitatively using methods which assess cell metabolic function and proliferation. The MTT assay is dependent on the intact activity of the mitochondrial enzyme, succinate dehydrogenase, which is impaired after exposure of cells to toxic surroundings. Briefly, the test involves the conversion of a tetrazolium salt 3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyl an insoluble formazan product, which can be quantified by spectrophotometry. Previous studies have shown that the processing method used for SEM causes precipitation of calcium carbonate polymorphs (Camilleri *et al.* 2004), resulting in artefacts and difficulty in viewing cellular morphology. In view of this, phosphate buffered saline was avoided in all parts of the experiment and substituted with phenol red-free medium to avoid precipitation of calcium phosphate crystals (Camilleri *et al.* 2005a).

Proliferation was determined using the Alamar Blue™ assay which is a redox indicator that can be used to quantitatively measure proliferation of cells (O'Brien *et al.* 2000). As the cells grow in culture, their metabolic activity maintains a reducing environment in the surrounding culture medium, whilst growth inhibition produces an oxidised environment. Reduction causes colour change of the Alamar Blue™ indicator from nonfluorescent (blue) to fluorescent (red). All the cements tested resulted in a lower proliferation rate compared with the control TMX. This had not been shown in previous studies evaluating the biocompatibility of GOPC (Abdullah *et al.* 2002), and GMTA (Koh *et al.* 1997, 1998, Mitchell *et al.* 1999, Perez *et al.* 2003). However, the results of this study support the findings of Haglund *et al.* (2003) who

showed that MTA had a cytotoxic effect on both macrophages and fibroblasts. No difference was observed in this study between the GMTA and WMTA in contrast to Perez *et al.* (2003) who showed that the grey variant was more biocompatible. In a recent study, Camilleri *et al.* (2004) showed cements cured for 28 days resulted in poorer cell growth. It was postulated that curing at 100% humidity might not have allowed the calcium hydroxide to go into solution thus affecting cell growth. In the present study the MTT showed that no toxic agent was leached from the cements. However, the frequent changing of culture medium may have disrupted the calcium hydroxide equilibrium of the solution leading to reduced cell attachment and a lower proliferation rate.

The use of a superplasticising admixture did not interfere with the biocompatibility of the cements, unlike the use of amorphous silica as a cement replacement which reacted with the calcium hydroxide produced resulting in reduced biocompatibility (Camilleri *et al.* 2005b). From the present study it can also be deduced that the addition of bismuth oxide was not directly related to the biocompatibility of the cements.

Conclusions

The setting time of Portland cement was reduced by excluding the gypsum in the final stage of the manufacturing process. No difference was observed between the grey and white MTA and addition of bismuth oxide did not interfere with the biocompatibility of the cements. Cell growth was poor when seeded in direct contact with the test cements. However, the calcium hydroxide produced during the hydration reaction was shown to induce cell proliferation.

Acknowledgements

The Commonwealth Scholarship Commission for funding; the Appleby Group, UK for grinding the prototype cement to specifications and for the delivery; the Malta Center for Restoration for access to equipment. Yoko Taniguchi for helping in the chemical analysis; Paula Coward, for the technical assistance and Professor Anton Buhagiar for the statistical analysis.

References

- Abdullah D, Pitt Ford TR, Papaioannou S, Nicholson J, McDonald F (2002) An evaluation of accelerated Portland cement as a restorative material. *Biomaterials* **23**, 4001–10.

- Camilleri J, Montesin FE, Papaioannou S, McDonald F, Pitt Ford TR (2004) Biocompatibility of two commercial forms of mineral trioxide aggregate. *International Endodontic Journal* **37**, 699–704.
- Camilleri J, Montesin FE, Brady K, Sweeney R, Curtis RV, Pitt Ford TR (2005a) The constitution of mineral trioxide aggregate. *Dental Materials* **21**, 297–303.
- Camilleri J, Montesin FE, Papaioannou S, McDonald F, Pitt Ford TR (2005b) The constitution and biocompatibility of a potential core build-up material. *Quintessence International* (submitted for publication).
- Haglund R, He J, Jarvis J et al. (2003) Effects of root-end filling materials on fibroblasts and macrophages in vitro. *Oral Medicine, Oral Surgery, Oral Pathology, Oral Radiology, and Endodontics* **95**, 739–45.
- Holland R, de Souza V, Nery MJ, Otoboni Filho JA, Bernabe PF, Dezan E Jr (1999) Reaction of rat connective tissue to implanted dentin tubes filled with mineral trioxide aggregate or calcium hydroxide. *Journal of Endodontics* **25**, 161–6.
- Holland R, de Souza V, Nery MJ et al. (2001) Reaction of rat connective tissue to implanted dentin tube filled with mineral trioxide aggregate, Portland cement or calcium hydroxide. *Brazilian Dental Journal* **12**, 3–8.
- Holland R, de Souza V, Nery MJ et al. (2002) Reaction of rat connective tissue to implanted dentin tubes filled with a white mineral trioxide aggregate. *Brazilian Dental Journal* **13**, 23–6.
- International Standard: Biologic Evaluation of Medical Devices (1992) *Part 5. Tests for Cytotoxicity: In Vitro Methods*. ISO 10993–5.
- Koh ET, Torabinejad M, Pitt Ford TR, Brady K, McDonald F (1997) Mineral trioxide aggregate stimulates a biological response in human osteoblasts. *Journal of Biomedical Materials Research* **37**, 432–9.
- Koh ET, McDonald F, Pitt Ford TR, Torabinejad M (1998) Cellular response to mineral trioxide aggregate. *Journal of Endodontics* **24**, 543–7.
- Lea FM (2001) *The Chemistry of Cement and Concrete*, 4th edn. London: Edward Arnold, pp. 177–86.
- Mitchell PJC, Pitt Ford TR, Torabinejad M, McDonald F (1999) Osteoblast biocompatibility of mineral trioxide aggregate. *Biomaterials* **20**, 167–73.
- Mosmann T (1983) Rapid calorimetric assay for cellular growth and survival; application to proliferation and cytotoxicity assays. *Journal of Immunological Methods* **65**, 55–63.
- O'Brien J, Wilson I, Orton T, Pognan F (2000) Investigation of the Alamar Blue (resazurin) fluorescent dye for the assessment of mammalian cell cytotoxicity. *European Journal of Biochemistry* **267**, 5421–6.
- Perez AL, Spears R, Gutmann JL, Opperman LA (2003) Osteoblasts and MG63 osteosarcoma cells behave differently when in contact with ProRoot™ MTA and white MTA. *International Endodontic Journal* **36**, 564–70.
- Saidon J, He J, Zhu Q, Safavi K, Spangberg LS (2003) Cell and tissue reactions to mineral trioxide aggregate and Portland cement. *Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, Endodontics* **95**, 483–9.
- Torabinejad M, White DJ (1995) *Tooth filling material and use*. US Patent Number 5,769,638; May 1995.
- Zhu Q, Haglund R, Safavi KE, Spangberg LS (2000) Adhesion of human osteoblasts on root-end filling materials. *Journal of Endodontics* **26**, 404–6.