INFLUENCE OF PLASMA PROTEINS ON SETTING CHARACTERISTICS OF MINERAL TRIOXIDE AGGREGATE-LIKE CEMENT

by

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A. ABSTRACT

Mineral trioxide aggregate (MTA) is a modified Portland cement (PC) based dental material containing bismuth oxide (Bi_2O_3) as a radiopacifier that is presently used as a sealing material in endodontic treatment. The present study investigated the influence of albumin, fibrinogen and γ-globulin, the three most abundant blood plasma proteins, both individually and in combination on the setting time, compressive strength and relative porosity of an MTA-like dental cement. The cement powder formulation was mixed with the liquid phase containing normal plasma protein concentrations or quarter, double or quadruple the normal plasma protein concentration as well as a mixture of all three proteins. Compressive strength and relative porosity were measured from 2 to 56 days and the initial setting times were determined using the Gilmore needles test. The set cement microstructure was examined using scanning electron microscopy (SEM) and energy dispersive X-ray spectroscopy (EDX). The compressive strength of all cements increased with time whereas strut density and relative porosity decreased. Fibrinogen and γ-globulin increased setting time with increasing concentration while albumin acted as an accelerant. For the MTA model mixed with all three plasma proteins, the setting times decreased as concentrations were increased.

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CHAPTER 1 - INTRODUCTION

1.1 *Basics of endodontics*

"Endodontics may be defined as the branch of dental science concerned with the study of form, function, health of, injuries to and diseases of the dental pulp and periradicular region and their treatment".[1] In endodontic practice diagnosis and treatment of dental pain and disease are considered to be fundamental in order for the tooth to function as a unit within the dental arch.[2]

Irritation or injury is often the cause of inflammation of the pulp, which can arise most commonly either from bacterial or mechanical irritation. Bacterial causes are from dental caries or decay, whilst mechanical irritation may be caused by tooth repositioning, operative procedures or trauma.[3]

1.1.1 Dental caries

Dental caries is the medical term for tooth decay or development of a cavity within a tooth. It is a process which arises on the tooth surface, where a microbial biofilm (dental plaque) develops over a period of time if not brushed or cleaned away. Streptococcus mutans is one of the bacteria which plays a significant role in the carious process, as it metabolises sugar in the food into acid, which then dissolves hydroxyapatite in the tooth structure.

The acid attacks the enamel which starts to break down forming a physical defect in the surface of the tooth. Following these food particles became trapped in the cavity which cannot be cleaned by brushing. For this reason a destructive process is more likely to progress and the acid dissolves the dentine and the patient may feel mild pain with sweet, hot and cold food or drink. This is due to movement of dentinal fluid caused by the change in temperature or osmotic pressure change inducing the nerve

fibres within the pulp chamber to elicit pain sensations. Treatment in this case is to remove decayed material from the cavity and then to replace lost tooth tissue with an appropriate restoration.[4]

If the tooth is left untreated, caries will ultimately reach the pulp, which will be infected by bacteria possibly causing an abscess. In this case the patient may feel sharp pain often being initiated by hot or cold drinks. In this situation, the tooth requires either endodontic treatment or extraction. [5]

1.1.2 Diagnosis

Diagnosis of the disease can be made from patient symptoms and clinical findings. Caries, trauma and abrasion will cause a patient to experience some pain, however, with the course of time this will eventually cease. This pain can be reignited by hot and cold drinks or sweets that stops on removal of the stimulus. Generally there will be no pain felt on biting and the periapical radiographic findings will usually be normal. The treatments for such cases is to remove the caries and restore the tooth with a suitable filling. The consequence of an untreated carious tooth, may lead the patient to develop irreversible pulpitis and cause spontaneous pain which could last from minutes to hours. The tooth will require either endodontic treatment or extraction. Ultimately, irreversible pulpitis (pulpitis that cannot recover) leads to pulp necrosis (death of the pulp) if left untreated will lead to periapical inflammation causing the patient to develop pain on chewing, typically eliciting no response to sweet, hot or cold stimulus. The treatment for pulp necrosis is the same as for irreversible pulpitis, root canal treatment (RCT) or extraction of the affected tooth.[3] Generally, the objective of RCT is to clean the infected pulp cavity and remove toxic debris and thereafter shape the root canal to enable it to receive a filling material.

The purpose of the filling material is to seal the whole root canal system from the periodontal tissues as well as the oral cavity. [3]

1.1.3 Preparation of the root canal system

There are generally only two approaches commonly recognized for root canal preparation, either orthograde (approaching via the crown of the tooth) or retrograde (approaching surgically via apical tissues). However, if it is not possible to prepare the entire length of the root canal then the surgical approach may be used to seal the canal system from the periapical tissues.[2]

RCT starts by gaining access to the pulp chamber through the occlusal or incisal surfaces and establishes the correct outline shape to gain access to the orifices of the root canals, using a high speed hand-piece that subsequently enables removal of the entire contents and allows inspection of the pulp chamber floor. The procedure of cleaning and shaping the root canals are carried out by hand as well as rotary instruments and bactericidal irrigation solutions such as 2.5% sodium hypochlorite solution are extensively used. The purpose of cleaning the root canals is to remove bacteria and any bacterial products or related irritants. This is achieved by intracanal irrigation and instruments such as rotary instruments or files which are positioned near the apex and then withdrawn while simultaneously scraping along the wall of the root canal when some binding is felt. A continuous tapering shape creates adequate space during canal enlargement to receive intracanal medicaments.[6] The procedure of irrigation is in essential cleaning and shaping the root canal as it washes away pulpal debris and dentine chips and also aids in lubricating endodontic instruments and facilitates the cutting action of the files. To establish the apical extent of instrumentation and the ultimate level of root canal filling, it is important to first establish exact working length of the canal which should be at the apical foramen.

This is done by means of a radiograph or by an electronic measuring device.[2] Prior to a root filling being inserted, the tooth must be asymptomatic and the root canal in a dry condition. The aim of root canal obturation is to immobilise any remaining bacteria with a hermetic seal in the canal from the coronal to the apical aspect of the tooth so that the remaining bacteria are in an environment where proliferation and surviving not possible. Apart from the apical seal which is essential, much emphasis has recently been placed on the need for a coronal seal as contamination can arise coronally particularly from saliva. Contamination of the root canal filling which could lead to failure may take place due to a poor coronal seal.[7]

1.1.4 Endodontic perforations

Endodontic perforations are a communication between the root canal space and the periodontal space caused iatrogenically while undertaking endodontic treatment. Perforations can have several different causes such as restorative defects, caries, or iatrogenic events (which are usually caused by a dentist's treatment of a patient) these can happen both during and after endodontic treatment. Whatever the cause may be, in the early stage of a perforation it cause inflammation, destruction of the periodontal tissues and loss of sulcular attachment after having invaded the supporting structures and ultimately the chance of successful treatment is reduced.[9] In the event of perforations a decision must be reached by the treating clinicians as to what course of treatment to take, either extraction or decide to leave the tooth in place for a surgical correction, nonsurgical treatment or a combination of the two. Treatment selection and prognosis are affected by a number of factors, including the length of time the perforation has been present in the tooth, severity of infection, size of perforation and relationship to the gingival margin. To prevent further loss of attachment and sulcular breakdown urgent attention must be given to repairing and

sealing the perforation hermetically. It is usually easier to deal with smaller perforations compared with larger ones. Furthermore, the chances of success in treatment are higher in the more apical perforations. [10]

Perforation repair can be achieved intracoronally and/or by an external surgical approach. The essential aspect in both approaches is to achieve a hermetic seal between the tooth and the material that is being used for the repair. This is determined by several important factors, namely, location and size of the perforation, operator skill and the physical and chemical characteristics of the repair material. [8]

An ideal endodontic repair material would adhere to the tooth structure, be dimensionally stable, fill canal irregularities and lateral canals (which are canals that lie perpendicular to the main root canal and can be found anywhere along the length of the root). The material also needs to have adequate sealing properties, be insoluble in tissue fluids, non-resorbable, radiopaque and demonstrate biocompatibility with human tissues.[2,11,12,13]

1.2 *Endodontic filling materials*

1.2.1 Materials used in endodontics

Amalgam had once been the material of choice for retrograde fillings as a consequence of its relatively easy handling, being radiopaque, non-soluble in tissue fluids and the sealing improves as the amalgam ages and corrodes, due to the corrosion products generating a barrier to fluid flow around the amalgam. However, amalgam has some disadvantages, namely dimensional instability and a slow setting time. Amalgam also needs an undercut in the cavity preparation for retention which reqires the removal of even more tooth tissue. Hard and soft tissues can also become stained by silver following amalgam use in the surrounding tissues which

can cause a tattoo.[14] Anderson *et al*. (1991) reported that microleakage of amalgam was reduced greatly by the usage of Amalgambond™ which is a self-cured bonding agent for the placement of direct restorations using amalgam.[15]

Another endodontic root filling material, zinc oxide-eugenol (ZOE) has the weaknesses of long setting time and high water solubility which were observed when used for retrograde fillings.[9] The use of ZOE as a root-end cement in periradicular surgery has not been extensively reported, but recent modifications of ZOE compounds, such as zinc oxide-eugenol with polymer reinforcement (IRM) and (Super EBA) provide a better seal. [16] Super EBA (32% eugenol and 68% ethoxy benzoic acid) is a zinc oxide-eugenol cement modified with ethoxybenzoic acid to decrease the setting time and increase its strength. Therefore it has several advantages over ZOE, is nonresorbable, radiopaque, has a neutral pH and low solubility. Even in a moist environment, Super EBA adheres to the tooth structure and is easy to use [16]. However, difficulty in placing and compacting may be experienced with Super EBA as it adheres to all surfaces and has a short setting time. [9]

Glass ionomer cement (GIC) is formed by the reaction of calcium–aluminosilicate glass particles with aqueous solutions of polyacrylic acid. GIC bonds physico-chemically to the dentine surface. Callis *et al*. (1987) showed that there was a cytotoxic effect in freshly mixed samples, although toxicity decreased with time.[17] On the other hand, MacNeil *et al*. (1987) reported that during the placement of GIC, its properties are affected when the root end is contaminated with moisture as the imbibitions of water changes the properties of GIC decreasing its ability to create an

adequate seal.[18] Use of acidic conditioners and varnishes improves marginal adaptation and adhesion of glass ionomer cements to dentine.[17]

Chong *et al*. (1991) reported using a light cured, resin reinforced GIC as a retrograde filling material and found that the reinforced GIC demonstrated the least microleakage due to a decreased moisture sensitivity, less curing shrinkage and a deeper penetration of the polymer into the dentine surface[19].

Calcium phosphate cement (CPC) is a mixture of two calcium phosphate compounds which was developed at the ADA-Paffenbarger Dental Research Center in the United States[5]. The most common variant is composed of dicalcium phosphate and tetracalcium phosphate reactants reacting isothermally to form a solid implant composed of hydroxyapatite when mixed with water [20]. The set cement consists of a crystalline material and porosity depends on the quantity of the aqueous phase. It is a radiopaque material and does not cause a continuous inflammatory or toxic reaction[22]. CPC has been shown to wet the dentinal walls well and the formation of hydroxyapatite as a product of the setting process is also considered advantageous as it is well tolerated by vital tissue.

In addition to plugging the apical foramen CPC has been shown to penetrate into the dentinal tubules in a scanning electron microscopic study.[21]

The compressive strength of CPC can be higher than 60 MPa and has been shown to maintain its form and volume over time. An *in vivo* study in monkeys found new bone developing immediately adjacent to the CPC[22]. The biomaterial CPC has been shown to have excellent biological properties and an acceptable mouldable property but appears to lack in mechanical properties therefore limiting its biological application.[23]

Calcium hydroxide cement consists of an activator paste made of calcium hydroxide, zinc oxide and zinc stearate in ethylene toluene sulphonamide, whilst the base paste is composed of calcium hydroxide cement contains calcium tungstate, calcium phosphate and zinc oxide in glycol salicylate. [24]

Calcium hydroxide cement is used in clinical application as an apical plug, root canal sealer and for both capping of an exposed pulp (direct pulp capping) and nearly exposed pulp (indirect pulp capping). The cement is also used as a protective barrier against external influences like bacteria and to encourage healing, so that beneath composite restorations the material does not interfere with the polymerisation of the composite. In addition, calcium hydroxide is routinely used as a dentine desensitizing agent in order to reduce dentine permeability, either by physical blockage of the tubule orifices and stimulation of secondary dentine.[25] The cements have an alkaline pH which varies from 11 to 12. For these cements a fast setting time varying from 2 up to 7 minutes and a compressive strength at one day of 26 MPa was found. The products of the cements greatly differ in their degrees of solubility in water and acid.[24]

1.2.2 Mineral trioxide aggregate (MTA)

Mineral trioxide aggregate (MTA) is a modified Portland cement containing a radiopacifier.[26] Torabinejad *et al*. (1993) developed MTA as a root-end filling material for periapical surgery and for the sealing of contact between the root canals and the surrounding tissues[27]. One of the major applications of MTA is its use as a retrograde filling material and for perforation repair. In the early 1990s scientists undertook an investigation into MTA to evaluate its use for root canal treatment. In 1998, the U.S Food and Drug Administration approved the use of MTA and it became commercially available as ProRoot MTA (Tulsa Dental Product, USA). Recently different types of

MTA have become available including grey and white forms and also from a different manufacturer, MTA-Anglus (Angelus Solucoes Odontologicas, Londrina, Brazil).[23]

1.2.2.1 Chemical and physical properties of MTA

The raw constituents of Portland cement (PC) are calcium oxide (CaO), silicon dioxide (SiO_2), alumina (Al_2O_3) and iron oxide (Fe_2O_3). When these raw materials are mixed, crushed and then heated up to 1400-1600^oC with added gypsum ($CaSO_4 \cdot 4H_2O$) to control the setting time, the resulting product consists of tricalcium silicate ($3CaO \cdot SiO_2$), dicalcium silicate ($2CaO \cdot SiO_2$), tricalcium aluminate ($3CaO \cdot Al_2O_3$) and tetracalcium aluminoferrite ($4CaO \cdot Al_2O_3 \cdot Fe_2O_3$)[23,28]. MTA is a mixture of 75 wt% PC, 20 wt% bismuth oxide (Bi_2O_3) radiopacifier to enable radiographic assessment of the cement and 5 wt% calcium sulphate as a setting modifier.[29,30] In addition, MTA has a smaller particle size compared with PC and is an expensive material[16] . Torabinejad *et al*. (1995) showed that within 4 to 6 hours a solid cement structure forms as a result of colloidal gel solidification after hydration of the powder.[31] Coomaraswamy *et al*. (2007) reported that the compressive strength of an MTA model system after ten days in an incubator at 37^oC was 36 MPa, the relative porosity was 23% and strut density 2.76 g/cm^3, compared with the commercial MTA which had a compressive strength of 33 MPa, relative porosity of 31% and a strut density of 2.74 g/cm^3.[30]

In comparison with zinc oxide eugenol (ZOE) and amalgam, MTA has a shorter setting time in a moist environment, better dimensional stability and also generates a better seal[32]. Peter *et al*. (2002) found that MTA has a significantly better adaptation to dentinal walls at the apex of the root than either amalgam or ZOE after submitting all three materials to a computer controlled simulated masticating device that produced an estimated 5 year equivalence of chewing cycles.[33]

1.2.2.2 Microleakage

The measure of success for any root canal treatment is the endodontic material's ability to adequately seal, as the vast majority of post-treatment endodontic problems are thought to arise as a consequence of unobturated and uncleaned apical areas of the root canal system, which then egress into the surrounding tissues and cause the whole root canal treatment to fail.

Torabinejad *et al*. (1993) has undertaken studies on the micro-leakage of MTA via *in-vitro* dye and fluid filtration methods comparing MTA with other traditional root canal materials in order to identify the advantages and disadvantages of MTA. MTA has been reported to have less micro-leakage than amalgam, ZOE or a conventional GIC when used for root-end restoration. [34,35]

In an investigation by Lamb *et al*. (2003) it was found that the minimal thickness for MTA to effectively seal the apical area should be at least 3mm.[36]

When used as a perforation repair material, MTA did not demonstrate any bacterial leakage during a 45-day evaluation while half of the amalgam-repaired perforation allowed bacterial penetration.[37]

Whilst providing the protection comparable with that of ZOE used for repair of furcation perforations, MTA also prevented micro-leakage to a greater extent than traditional materials when used for apical restoration. This has been shown by investigations using dye, fluid filtration and bacterial infiltration leakage methods. [38]

1.2.2.3 Biocompatibility

"Biocompatibility refers to the ability of a material to perform with an appropriate host response in a specific situation"[39]. It is well known that a biomaterial should cause no

harm to the recipient, which means the material should be non-toxic, non-irritant, non-allergenic and non carcinogenic. [40]

When placing materials in to the apex of the root canal it is never really possible to avoid contact with the surrounding tissue. So regardless of the dentist's precision some materials will be in contact with vital tissue. Therefore it must be ensured that the material does not cause any harm such as irritation or tissue necrosis.

Development of an intermediate layer of hard tissue deposition on the materials surface would be a positive response aiding in improving the quality of the apical seal. The possibility of recurrent infection as a consequence of the presence of bacteria at the apex of the tooth is a constant problem in endodontic treatment which warrants further research to identify a solution.

The cytotoxicity of amalgam MTA, ZOE, as well as positive and negative controls were investigated in human periodontal ligament fibroblasts after 24 hours[41]. It was observed that both the freshly mixed as well as in the 24 hour set samples the order of decreasing cytotoxicity was; amalgam, super-EBA, MTA.[40] Lin *et al*. (2004) found that human periodontal ligament fibroblasts mitochondrial dehydrogenase activity was not negatively affected by MTA.[42] Therefore MTA's use in the root apex should not elicit any negative response from the periodontal ligament fibroblast.

A normal morphology and growth of human periodontal ligament fibroblast was found with the scanning electron microscopy (SEM) analysis of periodontal ligament fibroblast as well as showing attachment to MTA surfaces set for 24 hours.

In an investigation carried out by Balto *et al*. (2003) with freshly mixed MTA samples, it was discovered that the human periodontal ligament fibroblasts had a round shape, lower density, showed signs of surface defects and lack of attachment to MTA. [43]

Haglund *et al*. (2003) reported that biocompatibility was shown by the set MTA with limited impact on cell growth after 72 hours and no observed effect on cell morphology was examined using phase-contrast microscopy.[44]

According to Baek *et al*. (2005), for periapical surgery of obturated canine with periapical lesion, MTA induced a favourable healing response of periapical tissue compared with amalgam and a ZOE preparation were verified radiographically and also in a double-blind histological evaluation. [45]

1.2.2.4 Clinical application of MTA

Pitt Ford *et al*. (1996) compared MTA with calcium hydroxide in pulp capping treatments in a monkey model, no inflammation was observed in the pulp in which MTA was used and 0.43mm continuous dentine bridge formed at five months between the pulpal tissues and the cement. These findings were in contrast to calcium hydroxide, where a third of all samples displayed dentine bridge formation and severe tissue inflammation, determined by histopathological analysis which was subjectively performed using light microscopy[46]. These results regarding comparison of MTA with calcium hydroxide were confirmed in a canine model which showed an improved tissue response in bridge thickness terms of formation of dentine bridges, whilst one third of the calcium hydroxide cases exhibited formation of dentine bridges and severe pulpal inflammation[47]. Human studies of direct pulp capping of teeth, comparing MTA and calcium hydroxide application after pulpal exposure in upper wisdom teeth at 7days and after 2 to 6months after treatment, were undertaken. The calcium hydroxide cases showed tissue inflammation and 0.15mm dentinal bridge

thickness at 6months. In contrast, MTA specimens exhibited no tissue reaction with a 0.28mm dentine bridge at 2months and 0.43mm dentine bridge formation at 6 months without signs of inflammation[48]. In a second study with 48 third molars after 30days the MTA group included 20 teeth with clinical normal pulpal status and 3 teeth with reversible pulpitis. In contrast, the calcium hydroxide group had 17 teeth with normal pulp status, 6 teeth had reversible pulpitis and one exhibited irreversible pulpitis[49]. A histological study of pulpotomy dressings was performed on extracted premolar teeth and concluded that MTA induced more continuous dentine bridge formation with no inflammation than calcium hydroxide 28 and 56days after pulpotomy treatment[50].

A clinical study by Chong *et al*. (2003) to determine the success rate of MTA and ZOE as root end filling materials was undertaken where 122 patients referred for endodontic surgery were treated with both ZOE and MTA preparations. No statistical differences between MTA and ZOE were found at both the 12- and 24-month recalls.[51]

Complete healing of repair sites took place in a study using MTA for root perforation repair in 16 cases. The cases consisted of 5 lateral root perforations, 5 strip perforations, three furcation perforations and three apical perforation, the study thus showed MTA being used in a range of perforation situations[52]. Though the initial results were promising, this study presented an incomplete view of MTA as no control group was included. However, several other studies showed that MTA used for treatment of horizontal root fractures, root resorption and furcation perforation were successful clinically and return of normal architecture observed radiographically. [8]

1.2.2.5 Indications for MTA use

MTA can be used as root end filling material as it has not been shown to cause significant inflammation in the short term and has shown formation of new dentine bridges leading to periapical regeneration in the long term. Secondly, MTA can be used in immature badly decayed teeth with open apices and with non-vital teeth as apical stops[53] and for perforation repairs. MTA is a hydrophilic material with a mineral substance similar to dentine which makes MTA a suitable repair material especially when applied directly after diagnosis of a perforation. Fourthly, MTA can be used as a repair for lateral root perforations. Furthermore, MTA has been shown to be an option for use as a coronal plug after obturation of the root canal has been completed. MTA must be applied into the prepared cavity before whitening of discoloured teeth or permanent restoration because of the MTA's sealing ability.[29] Finally, MTA can also be utilized as a pulp capping material and for pulpotomies and is the most common pulp treatment of the primary teeth before six years of age. The absence of micro leakage and bacterial contamination gives a better chance for the dental pulp to heal as MTA stimulates the formation of dentine bridges. [54]

1.3 *Modifying MTA*

During a surgical procedure in furcal and lateral root perforations, soft tissues will be exposed to the endodontic filling material used for repair. There will be for example blood which consists of a large number of plasma proteins. Any blood contact with a setting material is likely to affect the properties of the endodontic filling including its longevity. It is thus crucial to investigate the properties of MTA setting in the presence of these plasma proteins and in the present study albumin, fibrinogen and gamma globulin were chosen to represent typical plasma proteins found in the physiological enviroment.[55]

1.3.1 Albumin

Of all the plasma proteins, the globular albumin is the most abundant making up about 60% of the total serum protein and it is secreted entirely by the liver. Albumin plays an important role in maintaining the osmotic pressure of blood and is also involved in the transportation of fatty acids, bilirubin, pigments, ions and several amino acids. In addition to this, albumin regulates plasma concentrations of substances like tryptophan, calcium, steroid hormones and other substances by serving as an overflow carrier reservoir when the primary binding proteins become saturated.[56] The average level of plasma albumin is approximately 35000-45000 mg/ml and has a molecular weight of 69366.6 kDa (1 kDa = MW 1000) although this varies with gender and age; however, this level further decreases with increasing age. Clinically normal levels of albumin indicate good health whilst there is a reduction in plasma concentration in disease states. In patients suffering from nephrotic syndrome the concentration can decrease to levels below 35g/L.[56]

1.3.2 Fibrinogen

Another relatively abundant plasma protein is fibrinogen, which has a molecular weight of 340,000 kDa and is a large chain fibrous protein. It is easily precipitated as a salt and in the coagulation process a multitude of reactions occur whereafter fibrinogen, by the release of two fibrinopeptides, is converted into insoluble fibrin. The normal concentration of fibrinogen should be between 1900 to 3300 mg/ml, whilst most values are usually within 2200-2800 mg/ml. In contrast to albumin these values are not affected by age or sex. Hereditary afibrinogenemia (the total absence of fibrinogen) is a rare hereditary diseases in which sufferers have little or no

fibrinogen in their blood. The blood, as a consequence of the absence of fibrinogen, is incapable of coagulation, and hence excessive bleeding similar to that of a haemophiliac takes place. On the other hand, high levels of fibrinogen can be found in necrotic tissues and in malignant tissues.[56]

1.3.3 Gamma-globulin (γ-globulin)

Gamma-globulin is a widely used term for a class of plasma proteins which consists almost entirely of antibody proteins that arise from immunoglobulins (Igs). In the blood, the γ-globulin concentration is in the range of 7000-17000 mg/ml and has a molecular weight of 150,000 kDa. The immune system uses the protein in cases of invasion by unknown objects such as bacteria and viruses to identify and neutralise them.[55]

As a common feature γ-globulin has a very slow electrophoretic mobility in an alkaline buffer. The immunoglobulin concentrations that are given parenterally to humans for immune prophylaxis against infectious disease, or for passive antibody replacement in humoral immunodeficiency disease are also referred to as γ-globulin. γ-globulin is not a new term as its usage can be confirmed from early studies of immunology and the development of immonoprophylaxis. The proteins that were soluble in dilute salt solution and precipitated at half saturation with ammonium sulphate are known as globulins. The globulins were divided into two groups after it was discovered that were not all insoluble in water in the absence of salt as it was initially assumed. One group was called the ‘euglobulins’ (mostly immunoglobulin M, IgM) which followed the initial assumption and the other group was called ‘pseudoglobulins’ (mostly immunoglobulin G, IgG) which had the ability to dissolve even if salt was absent. [55,57]

CHAPTER 2 - MATERIALS AND METHODS

2.1 *Materials*

2.1.1 MTA model

The MTA model system was selected to enable laboratory production of a reproducible powder mixture with defined properties at an affordable price. The model system was modified by plasma protein addition in order to investigate the effect of plasma protein admixing on certain properties of the cement and how the possible changes may be explained. The cement mixed with all three plasma protein and the control without plasma protein were also investigated. The model cement consisted of 75wt% Portland cement (Blue Circle Mastercrete; LaFarge, Chilton, UK), 20wt% bismuth oxide (Bi_2O_3 ;Acros Organics, Loughborough, UK) and 5wt % calcium sulphate hemi-hydrate (Crystacal, Newark, UK).[30] All samples were hand-mixed using a spatula for 30s at a powder-to-liquid ratio (PLR) of 3g/ml with distilled water as the liquid phase.[30]

2.1.2 Protein addition

In this project the intrinsic approach directly applied the protein into the liquid component of the model cement system in order to observe the effect of proteins on the compressive strength and relative porosity of the cements. The extrinsic approach involved placing the cement into a mould where a defined volume of a protein containing solution was placed to mimic the conditions *in vivo*. A high concentration of either mixed plasma proteins or bovine serum, which represented body fluids, was placed into the mould so that the mould could represent the cavity site where MTA would be placed. Consequently, the body fluids would usually

combine with and encircle the cement mixture during and after cement setting similar to the clinical situation when the cement is placed into a cavity *in vivo*.

Three different plasma proteins were chosen to represent different protein conformations, namely albumin, γ-globulin and fibrinogen which are the most abundant plasma proteins in the human body. These plasma proteins were investigated to evaluate the effect of different protein concentrations and time periods on the properties of the cements: Albumin from bovine serum (A 2153, Sigma-Aldrich, UK), fibrinogen from bovine plasma (F 8630, Sigma-Aldrich, UK) and γ-globulin from bovine blood (G 5009, Sigma-Aldrich, UK). The cement powder mixtures were mixed with the liquid phase containing either the normal plasma protein concentration (normal 1 [protein]), quarter (0.25 [protein]), (2 [protein]) or quadruple the normal plasma protein concentration (4 [protein]) as well as a mixture of all three proteins. The cement paste was produced with the liquid phase containing distilled water with plasma proteins as shown in table 2.1.

Table 2.1 Variation of the plasma protein concentration in the liquid phase.

Protein	Normal plasma concentration[58,59] (mg/ml)	25% of normal plasma concentration (mg/ml)	400% of normal plasma concentration (mg/ml)
Albumin	50	12.5	200 (100 for 2 [albumin][a])
Fibrinogen	3	0.75	12
γ-globulin	10	2.5	40
Mixed plasma protein (Mixture of the three proteins)	albumin 50 fibrinogen 3 γ-globulin 10	albumin 12.5 fibrinogen 0.75 γ-globulin 2.5	albumin 100 fibrinogen 12 γ-globulin 40

[a] 2 [albumin] concentration represents 100 mg/ml

The mixture of the three proteins were used in the same concentrations, 0.25 [protein], 1 [protein] and 4 [protein], except for albumin which could only be admixed to a maximum of 2 [albumin] as the cement had a rubbery consistency at higher concentration and thus caused difficulties in placing the cement into the mould.

2.1.3 Cement sample production

A Gilson pipette was used to measure the liquid, thereafter the liquid was added to the MTA model, then the cement mixture was mixed thoroughly by hand with a spatula to produce the cement paste. The cement pastes were packed into a cylindrical polytetrafluroethylene (PTFE) mould (6 mm diameter, 12 mm height) and a glass slide was placed on top of the cement paste to provide a flat sample and to minimize drying out of the surface after filling the mould.

The extrinsic procedure adopted involved pipetting 60 microlitres of bovine serum or high concentration of mixed plasma proteins at the bottom of the mould and on the top of the cement paste following placement in the mould in order for the solution to surround and mix with the placed cement paste

Samples were extracted after 24 hours and then stored in distilled water and left to set for 2, 7, 28 and 56 days before testing in order to investigate how cement compressive strength and relative porosity developed with time. The mould was placed in an incubator at 37±1^{o}C for 24 hours to allow the cement samples (n>7) to set sufficiently to be extracted and stored in 20ml distilled water at 37^{o}C.[12,30,35]

2.2 *Methods*

2.2.1 Compressive strength testing

Both ends of the samples were examined for voids and damaged surfaces and flattend using abrasive papers (Silicone carbide paper 500) to a level where the ends were parallel and perpendicular to the long axis of the cylinder in order to prevent any movement of the samples and to ensure distribution of the applied force over the whole cross-section area when placed in the centre of the lower and upper plates of the mechanical testing machine. This was determined by measuring the length of the sample using a digital Vernier (4-5687, RS) in three different points from the sample and then taking an average. The same being repeated for the diameter of each sample. The weight was then measured to determine the wet density of each sample by using the formula below.

$$Wet\ density = \frac{Cement\ mass\ (g)}{\left(\left(\frac{Average\ width}{2}\right)^{2} \times \pi\right) \times Average\ height\ \times 1000\ \left(g/cm^{3}\right)}$$ [60,61]

The compressive failure load of the samples was determined using a Universal testing machine (Instron 5544, High Wycombe Bucks, UK) with a 10kN load cell at a crosshead speed of 1 mm/min.

To calculate the compressive strength the formula below was used, using the values for height, diameter, weight and failure load.

$$Compressive\ strength\ (MPa) = \frac{Failure\ Load}{(\frac{Average\ width}{2})^2 \times \pi}$$

After failure of samples under loading, all the sample fragments were weighed and then dried at room temperature in a dessicator for 2 to 4 weeks to remove water so that the dry mass of the fragments could be determined.

2.2.2 Density and relative porosity measurements

The strut densities of the powder mixture particles (around 1 g of reactant mixture) and fragments of the set cement sample (approximately 1 g of fragments from the compressive strength) were determined by the use of helium pycnometry (Accupyc 1330, Micromeritics, Bedfordshire, UK). Helium is used as it enters the smallest voids and pores in the sample and the pycnometer measures the volume of helium per unit weight of the sample and with the weight of the sample calculates the strut density of the sample. These measurements were performed in order to get an indication of the extent of the hydration reaction of the cement powder reactants in the set cement structure and therefore the presence of more soluble and denser unreacted powder in the structure.

The relative porosity (RP) of each cement sample was calculated using the dry density (mass/volume) of dried cylindrical sample fragments and the measured strut densities according to the formulae:

$$Dry\ density\left(g/cm^3\right) = \frac{Dry\ mass\ of\ \ fragments\ \ (g)}{Wet\ \ mass\ of\ \ fragments\ \ (g)} \times Wet\ \ density\ \left(g/cm^3\right)$$

$$Relative\ \ Porosity = 1 - \frac{Dry\ density\left(g/cm^3\right)}{Strut\ \ density\left(g/cm^3\right)}$$ [60,61]

2.2.3 Initial and final setting times

The initial and final setting times of the cement were measured using the Gilmore needles technique. The setting time is defined as the duration of time that elapsed from start of mixing to the point when the indentor needle of the Gilmore test failed to make an indentation. The initial setting time denotes the end of the workability of the cement paste after mixing and the final setting time indicates the hardening of the set mass. At a temperature of 23±1^{o}C, the cement paste was prepared and filled in the mould. A 113g needle with 0.21mm diameter and 454g needle with 0.11mm diameter were used to indent the cement and measurements were repeated every 15 minutes.[37, 62]

2.2.4 Scanning electron microscopy

The microstructure of the cement fragments was examined using scanning electron microscopy (SEM) (JEOL 5300LV SEM, UK) at 20kV in high vacuum mode. SEM images at magnifications of 500 and 1500 were captured for both smooth (the sample surface in-contact with the mould wall) and rough (the sample fracture surface) surfaces 2, 28 and 56 days after preparing the samples. Specimens were

sputter coated with gold for 2min, using a deposition current of 25mA, to generate a coating thickness of 15nm (K550x super coater, Emitech, UK) for dissipation of electrical charge and heat during examination.[30, 36, 37]

2.2.5 Energy dispersive X-ray spectroscopy (EDX)

EDX is an analytical technique used to identify the chemical composition of materials. The constituents of the control and the MTA model with 2 [albumin] were identified using high vacuum EDX at 20 kV (Philips XL-30 FEG Environmental SEM with Oxford Inca EDS). The thin uncoated samples were mounted on an aluminium stub and the spot analysis was performed 4 times in different places on the same sample.[38]

2.2.6 Workability

The workability was observed and evaluated subjectively with regard to the texture of the cements with different admixed plasma protein concentrations and compared with the control cement.

CHAPTER 3 - RESULTS

3.1 *Compressive strength*

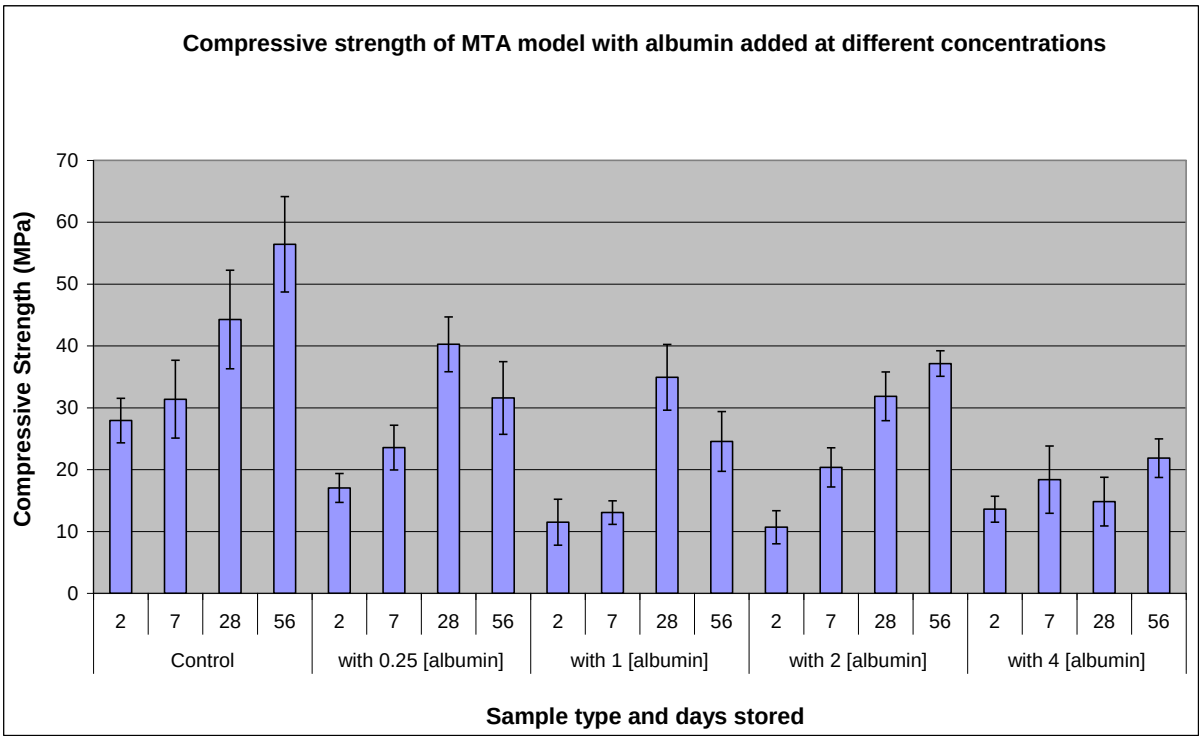

Fig 3.1.1 A graph showing the mean and standard deviation of the compressive strengths for the control and cements supplemented with different concentrations of albumin with time. As the albumin concentration increased the compressive strengths decreased.

The compressive strengths of the control samples increased with time stored in distilled water (Fig 3.1.1). The MTA model with 0.25 [albumin] increased in compressive strength after 2, 7 and 28 days with a further slight decrease on day 56. The MTA model with 1 [albumin] showed no notable difference between the compressive strength on day 2 and 7 but strength increased at day 28, whilst by day 56 there was a decrease in strength. The MTA model with 2 [albumin] showed a gradual increase in the compressive strengths from day 2 to day 56. The MTA model with 4 [albumin] showed no particular trend, with an increase after 7 days which then decreased after 28 days before a final increase after 56 days. In short, albumin supplements always caused a decrease in strength compared with the control, with

the lowest albumin concentration generating the highest strength of all albumin containing cements.

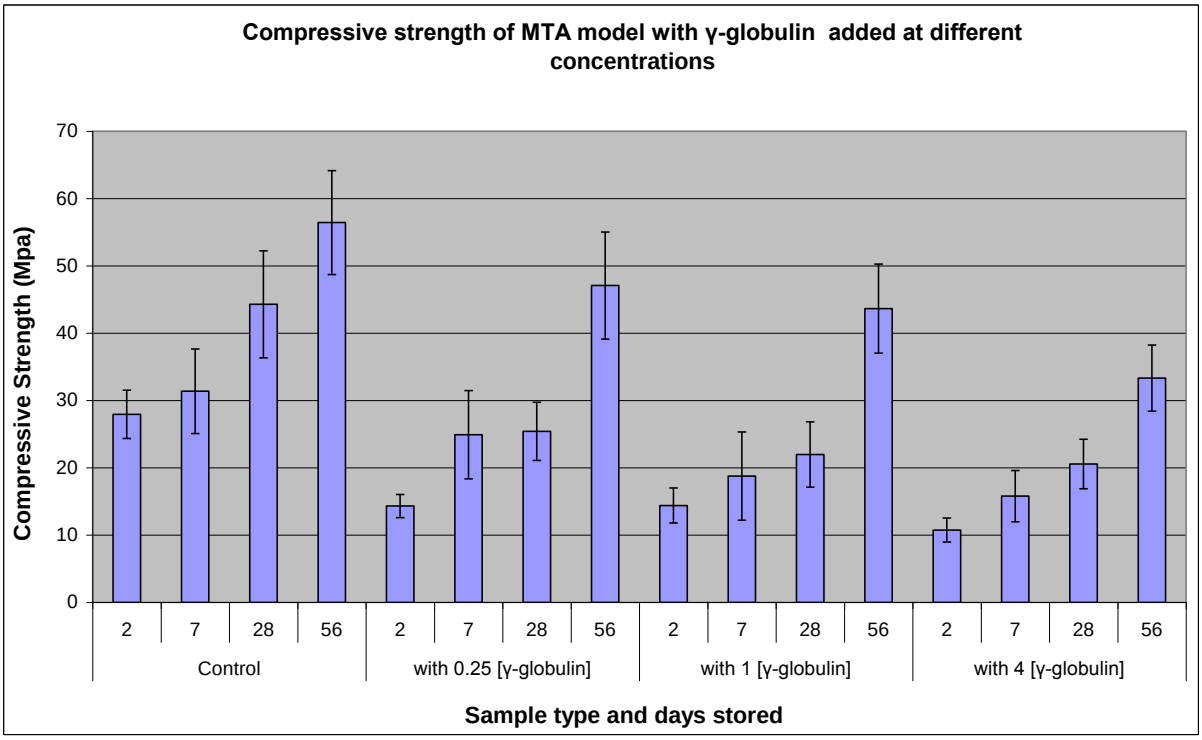

Fig 3.1.2 A graph showing the mean and standard deviation of the compressive strengths for the control and cements supplemented with different concentrations of γ-globulin with time. The strength was higher the lower the γ-globulin concentration.

The MTA model with 0.25 [γ-globulin] increased in compressive strength on days 2 and 7 (Fig 3.1.2). On days 7 and 28 the compressive strengths were similar although there was a marked increase in compressive strength on day 56. The MTA model with 1 [γ-globulin] and 4 [γ-globulin] increased in compressive strengths from day 2 to 56, the same trend was observed in the control. Generally the strength was higher the lower the γ-globulin concentration.

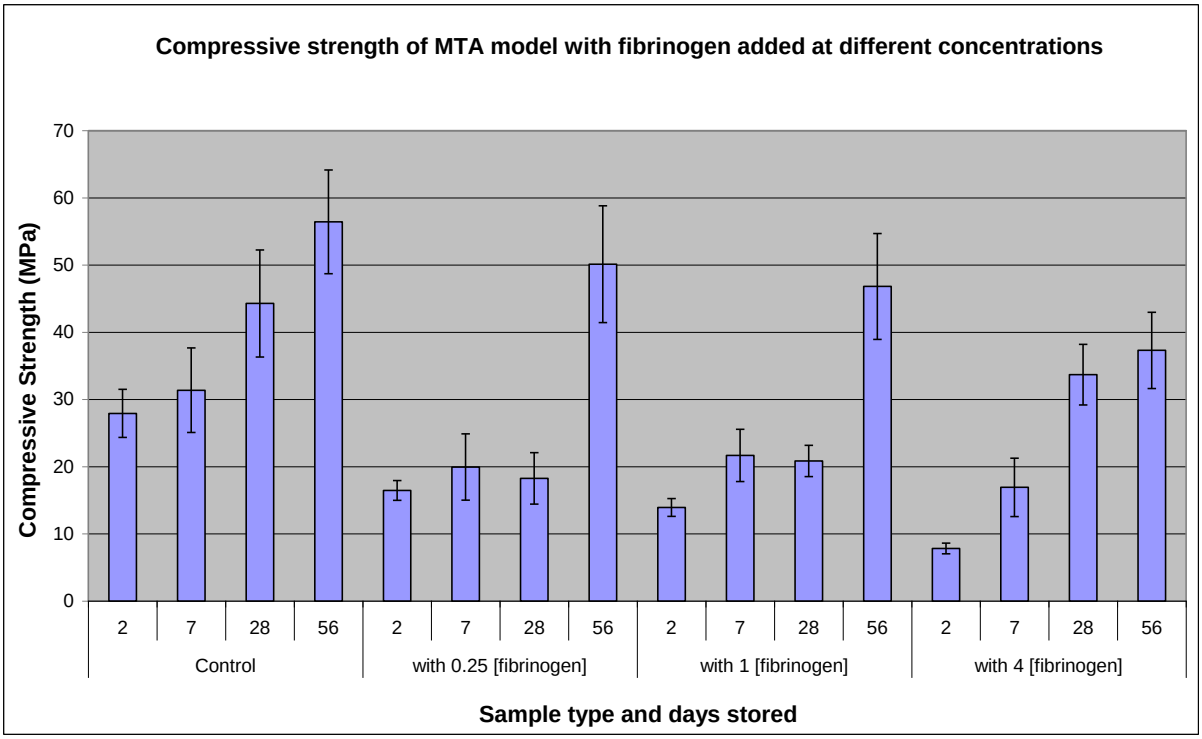

Fig 3.1.3 A graph showing the mean and standard deviation of the compressive strength for the control and cements supplemented with different concentrations of fibrinogen with time. Fibrinogen supplements always caused a decrease in compressive strength at all time points when compared with the control.

The MTA model with 0.25 [fibrinogen] and 1 [fibrinogen] showed a similar pattern of compressive strength development especially between days 2 and 28 (Fig 3.1.3) in addition on days 56 both concentrations showed a sharp increase. The MTA model with 4 [fibrinogen] showed a gradual increase in the compressive strengths as also appeared to be the trend for the control. The higher the fibrinogen concentration, the lower the initial (2 days) and final compressive strengths (56 days). In short, fibrinogen supplements always caused a decrease in compressive strength at all time points when compared with the control.

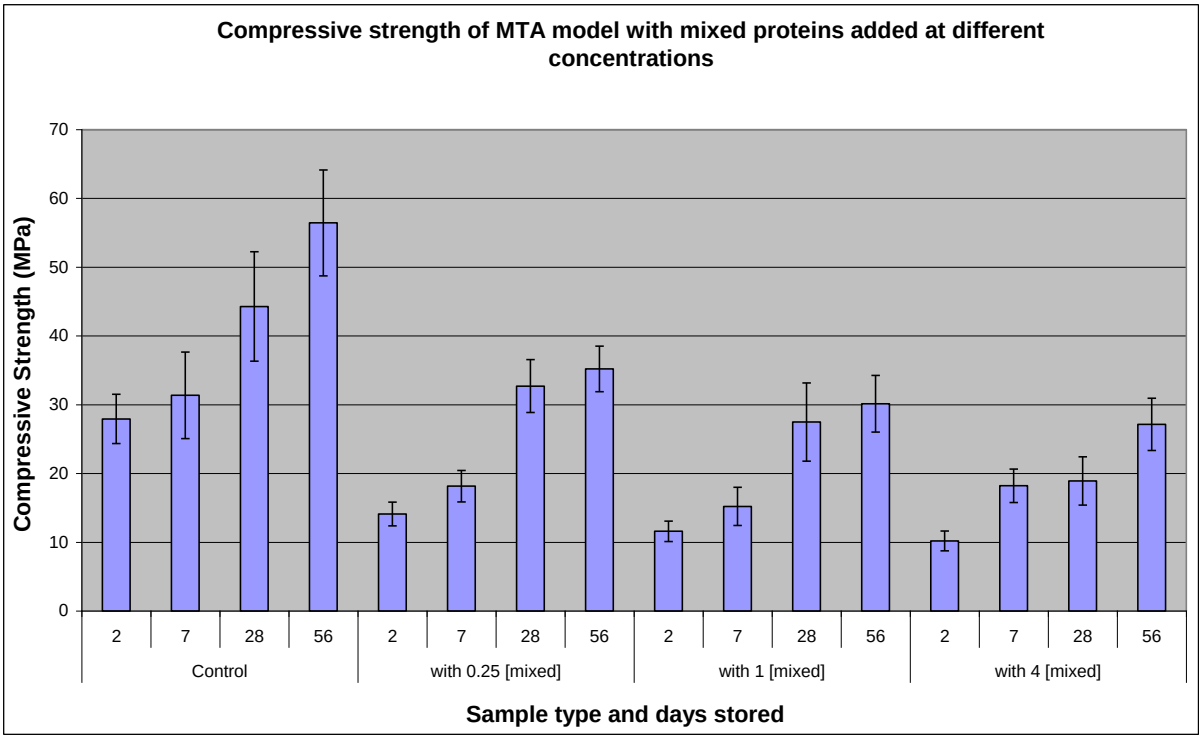

Fig 3.1.4 A graph showing the mean and standard deviation of the compressive strengths for the control and cements supplemented with different concentrations of mixed proteins with time. The higher the mixed proteins concentration, the lower the compressive strength.

The MTA model with 0.25, 1 and 4 [mixed protein] increased in compressive strength from days 2 to 56 (Fig 3.1.4) which were similar to the trend of the control. However, between 28 days to 56 days there was a noticable increases in compressive strengths for control and 4 [mixed protein] compared with a slight increase for 0.25 and 1 [mixed protein], so the higher the mixed proteins concentration, the lower the initial (2 days) and final compressive strengths (56 days).

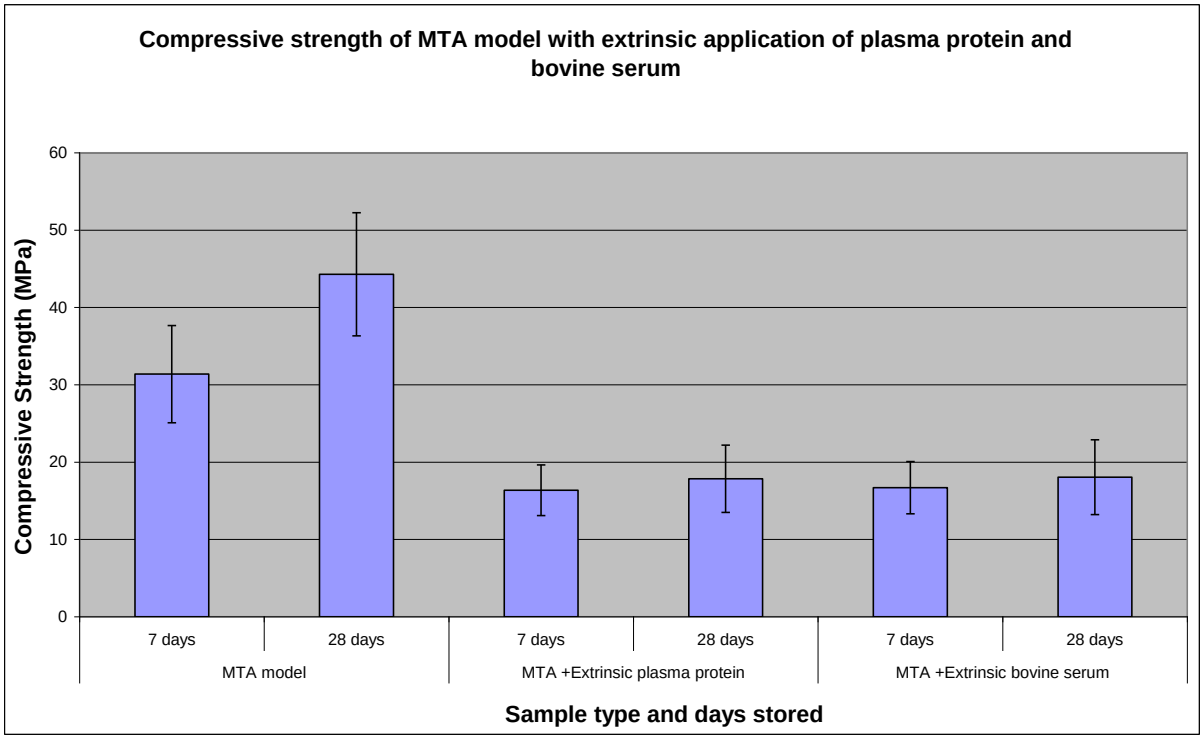

Fig 3.1.5 The mean and standard deviation of compressive strengths with time for the control, cement with extrinsic application of plasma protein 4 [mixed protein] and bovine serum. Extrinsic application of 4 [mixed protein] and bovine serum always caused a substantial decrease in compressive strength when compared with the control.

The compressive strengths of the control samples increased with time (Fig 3.1.5). The MTA model with extrinsic application of 4x [mixed protein] increased in compressive strength after 7 and 28 days. The MTA model with bovine serum showed a similar pattern of compressive strength development. In short, extrinsic application of 4 [mixed protein] and bovine serum always caused a substantial decrease in compressive strength when compared with the control.

3.2 *Relative porosity*

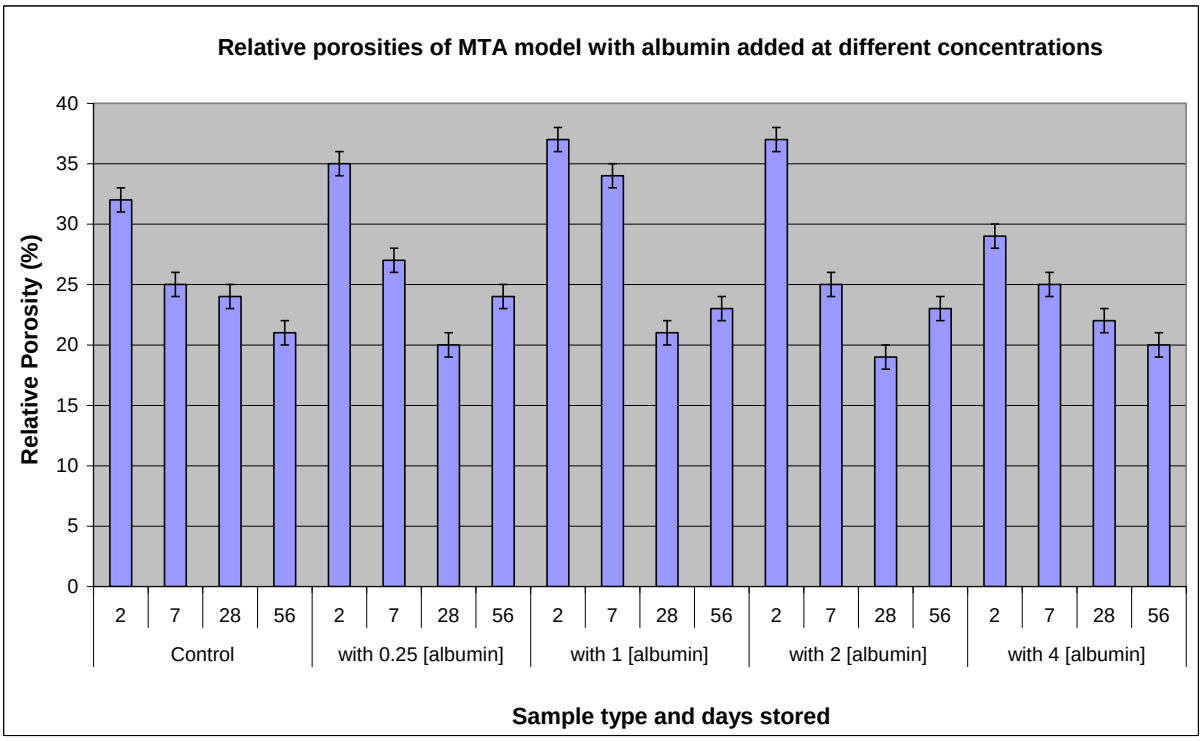

Fig 3.2.1 Mean of the relative porosities for the control and cements supplemented with different concentrations of albumin with time. The relative porosities generally trend to decrease with setting time, only for 0.25, 1 and 2 [albumin] the relative porosities increased after 56 days. The minimum error of the method is represented by the error bars.

The relative porosities of the control samples decreased gradually with time (Fig 3.2.1). The MTA model with 0.25, 1 and 2 [albumin] showed a steady decrease in the relative porosities on days 2, 7 and 28 whilst on day 56 there was an increase. The MTA model with 4 [albumin] showed a gradual decrease in porosity with time. However, on day 2 the relative porosities of the MTA model with 1 and 2 [albumin] were notably increased compared with the control. In general the relative porosities decreased with setting time, only for 0.25, 1 and 2 [albumin] did the relative porosities between 28 and 56 days.

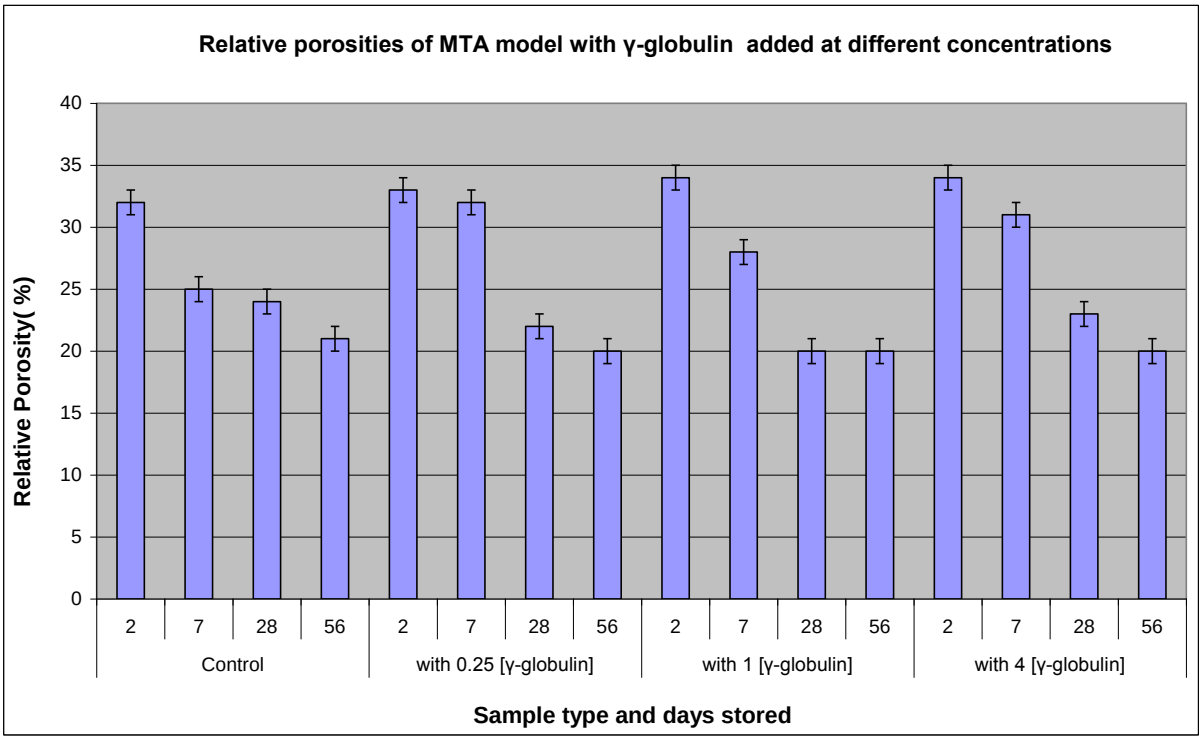

Fig 3.2.2 Mean of the relative porosities for the control and cements supplemented with different concentrations of γ-globulin with time. The relative porosities decreased with increasing setting time. The minimum error of the method is represented by the error bars.

The relative porosity of the MTA model with 0.25, 1 and 4 [γ-globulin] decreased from days 2 to 56 (Fig 3.2.2) which were similar to the trend of the control, whilst, there was no change in relative porosity between day 28 and 56 for 1 [γ-globulin]. Relative porosities for γ-globulin containing cement decreased more slowly than for the control after 7 days and after 56 days relative porosities were similar for all cements. In short, the relative porosities decreased with increasing setting time.

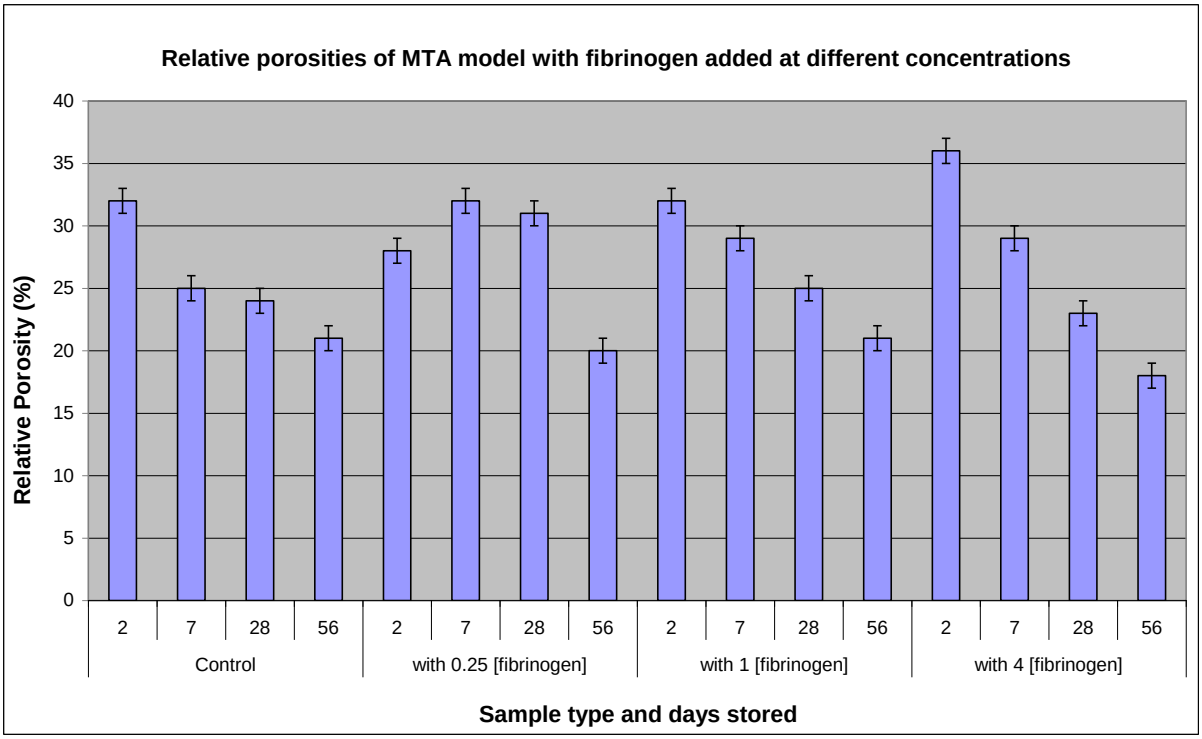

Fig 3.2.3 Mean of the relative porosities for the control and cements supplemented with different concentrations of fibrinogen with time. As a trend, the relative porosities decreased with setting time, except for 0.25 [fibrinogen] which showed an initial increase in the 2 days stored sample when compared with the control and other fibrinogen samples. The minimum error of the method is represented by the error bars.

The MTA model with 1 and 4 [fibrinogen] decreased in relative porosity from days 2 to 56 (Fig 3.2.3) which was similar to the trend of the control. However, on day 2 for 0.25 [fibrinogen] the relative porosities were less than those observed on day 2 for the other concentrations. The higher the fibrinogen concentration, the higher were the initial relative porosities. So, the relative porosities decreased with setting time, except for 0.25 [fibrinogen] which showed an initial increase in the 2 days stored sample when compared with the control and other fibrinogen samples.

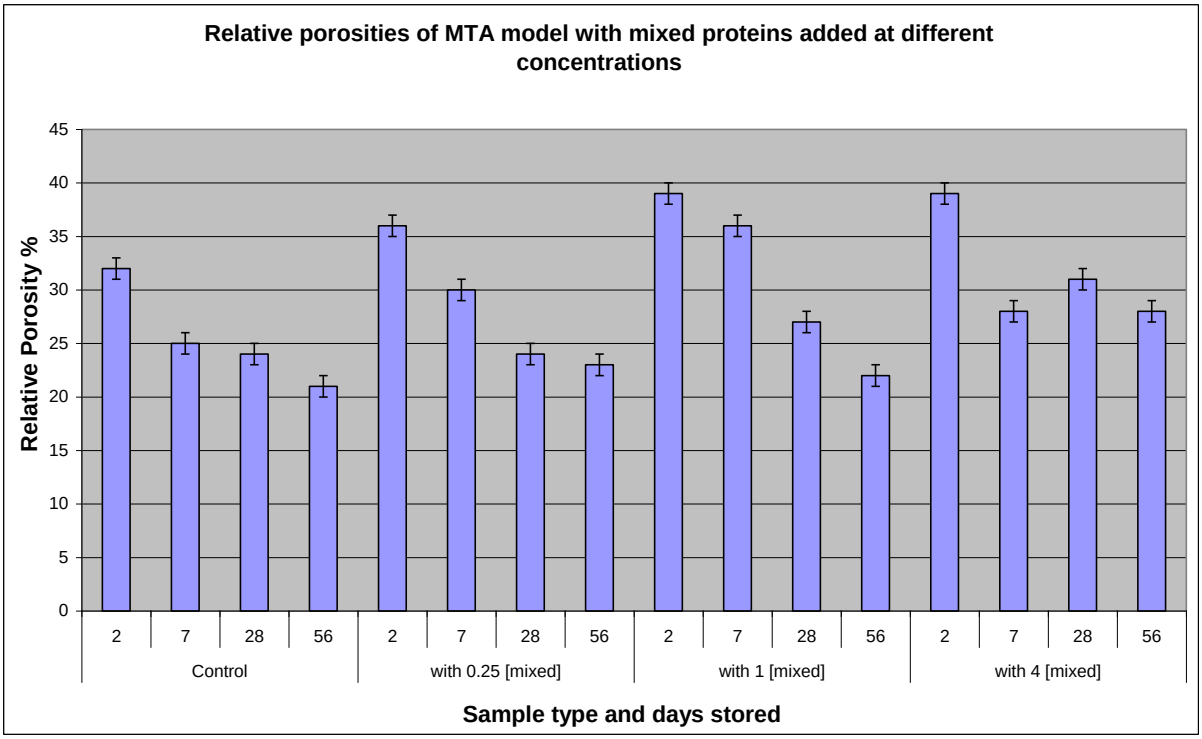

Fig 3.2.4 Mean of the relative porosities for the control and cements supplemented with different concentrations of mixed proteins with time. Relative porosities decreased with setting time, only for 4 [mixed protein] the relative porosities remained constant between 7 and 56 days. The minimum error of the method is represented by the error bars.

The MTA model with 0.25, 1 and 4 [mixed protein] decreased in relative porosity from days 2 to 56 (Fig 3.2.4) which were similar to the trend demonstrated by the control. Relative porosities decreased with setting time, only for 4 [mixed protein] the relative porosities remained constant between 7 and 56 days. In short, the higher the concentration of mixed proteins, the higher the relative porosities.

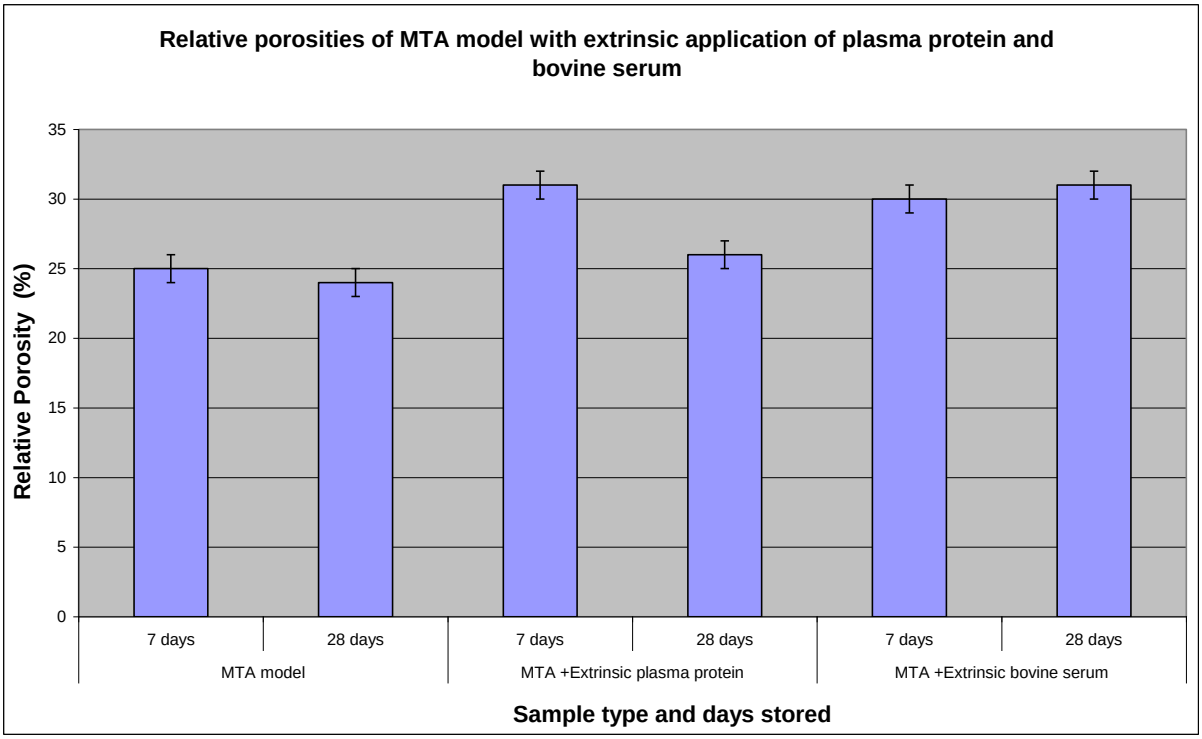

Fig 3.2.5 Mean of the relative porosities for the control, with extrinsic application of plasma protein 4 [mixed protein] and bovine serum with time. For the control and bovine serum there was practically no change whilst for the sample with extrinsic application of 4 [mixed protein] there was a decrease in the relative porosity. The minimum error of the method is represented by the error bars.

The relative porosities of the samples of control and MTA model with extrinsic application of bovine serum demonstrated roughly the same level between 7 and 28 days (Fig 3.2.5). The MTA model with extrinsic application of 4 [mixed protein] showed a decrease in the relative porosities between 7 and 28 days. In short, for control and bovine serum there was practically no change whilst for the sample with extrinsic application of 4 [mixed protein] there was a decrease in the relative porosity.

3.3 *Strut density*

The measured strut densities for the controls and with admixed plasma proteins ranged between 2.8 g/cm^3 for samples incubated with distilled water for 2 days to 2.5 g/cm^3 for samples incubated for 56 days, whilst the strut densities of the MTA model with 4 [albumin] stored for 56 days was still 2.68g/cm^3 and notably higher when compared with the controls and other cements with admixtures.

3.4 *Setting time*

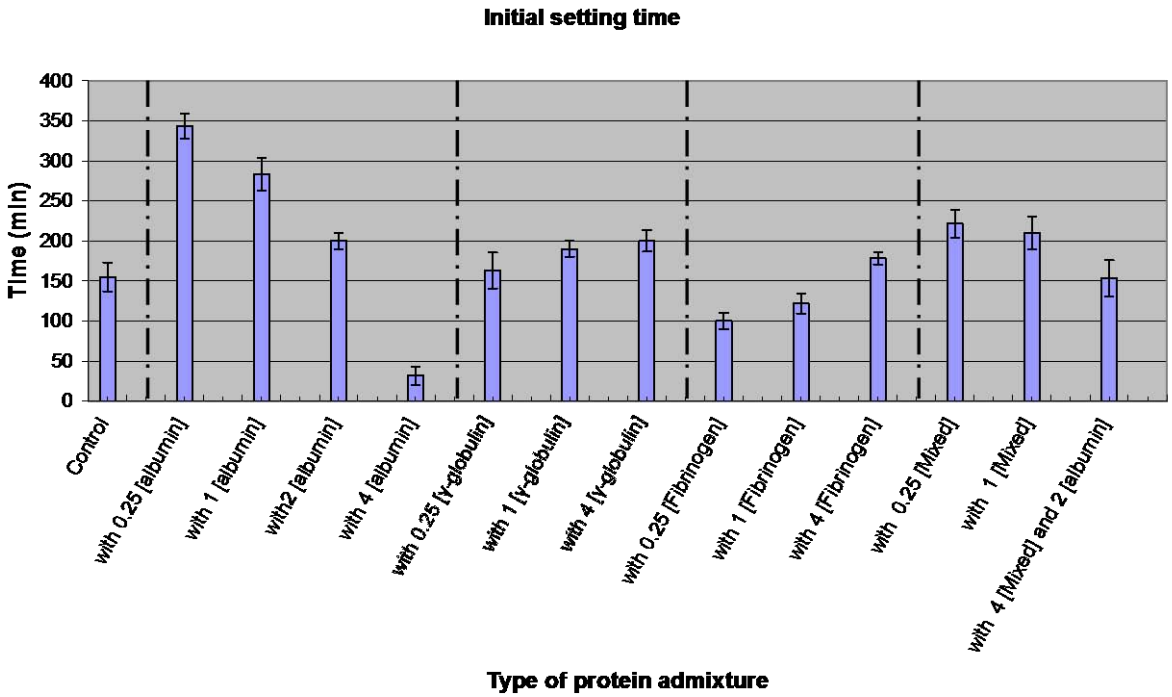

Fig 3.4.1 A graph showing the mean and standard deviations of the initial setting times for the control and the cements supplemented with different concentrations of plasma proteins added to MTA. Fibrinogen and γ-globulin increased setting time (retardants) while albumin acted as an accelerant with increasing concentration.

There was a notable increase in the initial setting time for MTA with 0.25 [albumin] compared with the control (Fig 3.4.1). However as the albumin and mixed proteins concentration increased the setting time decreased and 4 [albumin] demonstrated the fastest setting compared with all other plasma proteins. On the contrary, as the concentration increased for γ-globulin the setting time also increased.

There was a decrease in the initial setting time for fibrinogen admixtures compared with the control. With increasing concentration, fibrinogen and γ-globulin increased setting times while albumin decreased it. So increasing γ-globulin and fibrinogen concentrations acted as retardants while albumin was acting as an accelerant.

3.5 *Scanning Electron Microscopy*

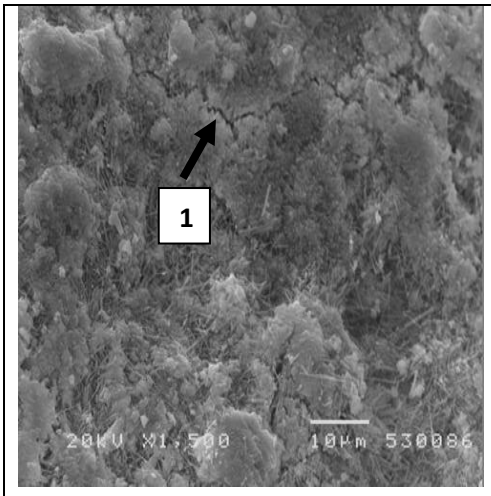

Fig 3.5.1 SEM secondary electron micrographs showing MTA model (control) set for 2 days that represents capillary channels

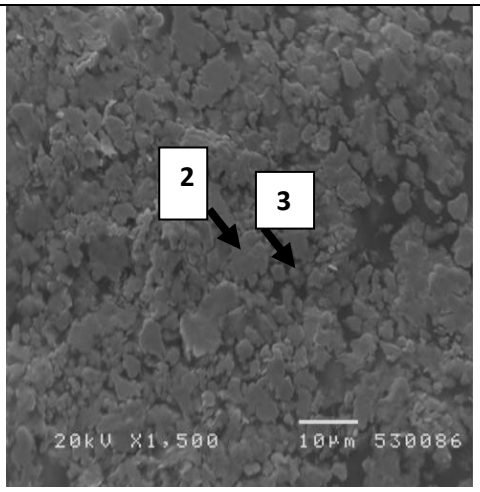

Fig 3.5.2 SEM secondary electron micrographs showing ٢ [albumin] set for 2 days that shows small irregular particles interspersed by large particles

The SEM secondary electron micrographs showed small irregular particles interspersed with large particles as shown by arrows 2 and 3 in fig. 3.5.2 and capillary channels as shown by arrow 1 in fig. 3.5.1.

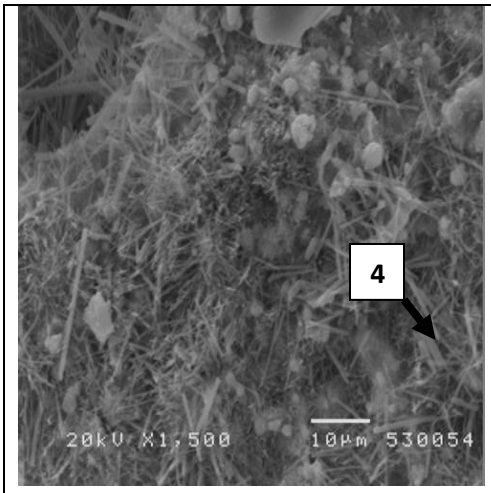

Fig 3.5.3 SEM secondary electron micrographs showing MTA model (control) set for 56 days that illustrates formation of relatively small crystals

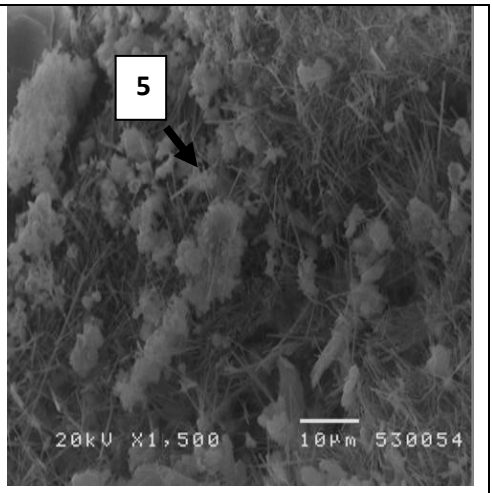

Fig 3.5.4 SEM secondary electron micrographs showing 2 [albumin] set for 56 days shows that the crystals were clustered together

The SEM secondary electron micrographs showed the formation of relatively small crystals, depicted by arrows 4 and 5, that were clustered together figure (3.5.3 and 3.5.4)

In summary the microstructures of the control and samples containing different concentrations of the plasma protein appeared relatively similar at days 2, 7, 28 and 56, except that on day 56 there were crystal precipitations observed on the SEM micrographs.

3.6 *Energy dispersive X-ray spectroscopy*

The EDX analysis for the MTA model and 2 [albumin] incubated for 56 days in distilled water indicated the presence of calcium, bismuth, sulphur, silicon, oxygen, aluminium and carbon in the smooth surface of the sample. By calculating the sum of the molecular weight of calcium sulphate and comparing it with the EDX results

reguarding percentage in weight, the needle-like crystals seen with SEM on the surface of the MTA model sample were determined to be chemically similar to calcium sulphate dihydrate (gypsum). The total molecular weight of gypsum is 172 consisting of 23 wt% calcium, 19wt% sulphur and 56wt% oxygen. The EDX measurements showed 54wt% calcium, 10wt% sulphur, 32 wt% oxygen and the remainder being silicon and aluminium. Calcium can stem from other sources and is thus abundantly present in the cement, whereas sulphur is only present in the calcium sulphate. The sulphur to oxygen ratio (≈ 1:3) of the precipitates was therefore used to identify the composition of the precipitates. The theoretical sulphur to oxygen ratio of gypsum by weight is 1:3. The precipitates were therefore identified as gypsum crystals.

3.7 *Workability*

The workability of the cements compared with the control was subjectively observed for the various plasma proteins concentration. The cement pastes that were mixed with 1 and 4 [fibrinogen] were very sticky and viscous. The paste mixed with 4 albumin concentration had a rubbery consistency that was difficult to work with and place into the mould. However, the cement paste mixed with 0.25 and 4 [γ-globulin] were more fluid especially at high concentrations. On the other hand, the cement pastes mixed with different types of plasma proteins at different concentration were more workable.

CHAPTER 4 - DISCUSSION

4.1 *Compressive strength and relative porosity*

Relative porosity is a measure of the amount of water in the cement pore system [60]. There are three possible sources of porosity in the cement system, either from the unreacted water left from the formation of the cement paste, as a consequence of partially dry powder agglomerates (dry spots) and air bubbles which cause air to be trapped in the cement paste. The Portland cement manufacturing industry use compressive strength as an indicator for the durability and thus potential longevity of cements [63]. There is a relationship between compressive strength and relative porosity which was first proposed by Takashi *et al.* (1997)[64] after a study on hardened Portland cement-based systems. It was later discovered that this relationship was also valid for other calcium phosphate based-cement systems [65,66]. The equation below demonstrates this relationship: [30]

$$CS = \sqrt{\frac{E_0 R}{\pi \cdot c}} \cdot e^{-const \cdot RP}$$

Where CS is the compressive strength, E_0 is the modulus of zero porosity material, R is the fracture surface energy, c is the critical flaw size and RP is the relative porosity.

The relationship between compressive strength and relative porosity is inverse, as when the relative porosity decreased the compressive strength increased and this was the case for most of the cement samples, as addition of different plasma

proteins to the liquid phase decreased the compressive strength and increased the relative porosities of the cement. The plasma proteins influenced the properties of the MTA model, where the proteins may have covered or coated the powder surfaces by adsorbing to the surface and this may be the reason for the altered properties of the MTA model. Studies by Xie J *et al*. (2001) have shown that albumin blocked the interaction of Ca^{2+} and PO_4^{-3} with water molecule causing the dissolution and precipitation of calcium phosphate cement and thus affecting the properties of calcium phosphate based-cement systems[67]. PC setting can potentially be blocked by the action of albumin due to it having a similar constituents in the calcium ion. To further investigate regarding MTA and show if adsorption of the proteins have taken place, a calcium selective electrode could be used. Albumin binds calcium and therefore reduces the availability of calcium ions and thus slows down the reaction. Therefore if the measurement of the dissolution of particles with time is recorded, a comparison could be made to indicate if something goes into solution slower or faster depending on what is added to it. If the rate of dissolution of a normal solution is first timed, it can act as a base value to compare the dissolution rates with additions to the solution and for example whether adding albumin slow down the reaction as in the case of adding albumin. This will cause an increase in dissolution time as the albumin binds to the calcium and so will indicate that albumin binds to calcium.

For albumin, γ-globulin and fibrinogen additions, the compressive strength decrease was associated with an increase in relative porosities, this behaviour may be explained by protein molecules coating the powders. These molecules then may have occupied additional space in the cement paste and in turn created pores in the hardened cement and thus led to a slight increase in the porosity and an increase in the number of defects of the cement matrix. However, this explanation is not

supported by the SEM as no evidence of porosity was observed even at a magnification of X1500, although microporosity would not necessarily be visible at this magnification. The lowest concentration used for all three plasma proteins was 0.25 [protein] and showed a small decrease of compressive strength compared with the control and high relative porosities, whereas the higher concentrations of plasma proteins addition showed more obvious decreases in compressive strength compared with the control. Compressive strength increased for both the control sample and the other concentrations of plasma proteins containing cement with time. However, by day 56 there was a decrease in the compressive strength of 0.25 and 1 [albumin] samples with an obvious increase in relative porosities, with time the formation of pores takes place and leads to the decrease in compressive strength with both albumin additions as seen (Fig 3.1.1 and Fig 3.2.1), similarly as found with calcium phosphate cements by Chauhan *et al*. (2006). It was found that the compressive strength of a calcium phosphate cement with albumin and fibrinogen concentration of 0.1wt%, 0.5wt%, 1.0wt% and 10.0wt% decreased with increased concentration of albumin and fibrinogen, this was accompanied by increases in relative porosities. A decrease in compressive strength was also shown when the study was conducted in the presence of albumin, a 0.1wt% albumin concentration lead to a reduction of compressive strength by 11% and for albumin concentrations of 0.5wt%-10wt% compressive strength decreased by over 40%. Similar decrease of compressive strength and increase of relative porosity were seen with fibrinogen. [57]

The extrinsic application of the MTA model into bovine serum and 4 [mixed protein] showed a similar pattern of compressive strength development. Extrinsic application caused a substantial decrease in compressive strength and increase in relative porosities.

The explanation for this behaviour could be due to high porosities, which makes the cement less homogeneous and introduces a high number of flaws and air bubbles which was observed on the fractured surface. These flaws may expand and therefore facilitate crack formation in the cement, causing a decrease in the compressive strength.

4.2 *Setting time*

The addition of plasma proteins caused obvious changes in the setting times of the cement mixture. Addition of 0.25 [albumin] increased the initial setting time, this may be because at low concentrations the albumins coats the particles preventing them from reacting, therefore slowing the reaction and increasing the setting time. But when the concentration increased the setting times decreased. This may be because albumin molecules acted as an adhesive material gluing the cement particles together and thus decreasing the setting time, or the decrease of initial setting time compared with the control that may have been caused by denaturation of albumin due to the high pH. However there is no direct evidence in current studies so further investigation in this field is required to address this matter. This could be carried out by antibody staining of albumin bound to MTA cement particles.[68]

Additions of fibrinogen appeared to decrease the initial setting time compared with the control, but when the concentration increased the setting times increased. This may be because fibrinogen is a fibrous protein with a spiral shaped structure and might connect the particles at low concentration and when the fibrinogen concentration increased the fibrinogen molecules around the powder particles may have prevented wetting of the powder particles and thereby delayed the setting time.

Addition of γ-globulin showed that as the concentration increased the setting reaction was delayed. The reason for this may have been due to γ-globulin being a large globular structure which allowed a greater surface interaction with the reactant powders and thus preventing the wetting of the powders and therefore the setting reaction was retarded as the concentration increased.

For the MTA model mixed with three plasma proteins, the setting times appeared to be a combination of these effects. At high concentration albumin dominated thus leading overall to a decrease in setting time when compared with the control.

4.3 *Strut density*

The specific densities for all cements ranged between 2.8 g/cm^3 for samples incubated with distilled water for 2 days to 2.5 g/cm^3 for samples incubated for 56 days. A decrease in the strut densities was observed as the setting time progressed, as more water became chemically bound to the set material in the water consuming hydration reaction, subsequently compressive strength increased and porosity decreased with time. The strut density of the control cements along with all the other cements showed similar patterns of development with time when compared to each other. However, higher strut densities of the MTA model with 4 [albumin] for 56 days were observed when compared with the control and other admixtures. This could have been a consequence of the high albumin concentration preventing hydration of the cement particles and therefore interfering with the setting reaction even after 56 days.

4.4 *SEM*

The microstructure of MTA model samples with or without protein addition appeared similar which indicated that there was no apparent change in the structure due to the addition of protein for cements with and without protein addition, the structure became more dense with increasing setting time for all cements.

4.5 *EDX*

The precipitate formed by EDX could be identified as the calcium sulphate dihydrate (gypsum). However, it could not be determined whether this crystal precipitation material was generated during the period that the samples of the MTA model were in solution or while the samples were in the process of being dried.

CHAPTER 5 - CONCLUSIONS

This study investigated the influence of three plasma proteins on setting and material properties of an MTA-like cement. In the investigation, it was confirmed that the decrease in compressive strength was inversely proportional to an increase in relative porosity of the cement. It showed that the plasma proteins and an increase in their concentration lead to increased porosity of the cement matrix and thus a decrease in compressive strength. For albumin, fibrinogen and γ-globulin additions, the compressive strength decreased with increasing concentration which weakened the cement structure. With respect to the setting time, an increase in γ-globulin and fibrinogen concentration retarded the setting while albumin acted as an accelerant. The effect of the mixed protein addition appeared to be a combination of the individual proteins. The extrinsic addition of bovine serum and the high concentration of mixed proteins to the MTA model, caused a substantial decrease in compressive strength and increase in relative porosities. It was observed using SEM and determined by EDX that the needle-like crystals on the surface of the MTA model sample were chemically similar to calcium sulphate dihydrate (gypsum).

The study indicated that the three plasma protein admixtures tested evidently had a substantial effect on the material properties of a Portland cement based cement and as such have to be taken into account when developing and investigating such a material.

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