Original Article International Journal of Dental Materials 2021; 3(3) ISSN:2582-2209

In vitro hydroxyapatite formation of a tetracalcium phosphate and anhydrous dicalcium phosphate based dentine desensitiser: TRIS buffer vs artificial saliva

Tomas Duminis[1,*], Saroash Shahid[1]

[1]Centre for Oral Bioengineering, Institute of Dentistry, Queen Mary University of London, United Kingdom.

INFORMATION

Article History

Received 5th *May 2021*

Received revised *17th June 2021*

Accepted *1st July 2021*

Available online *1st August 2021*

KEYWORDS

Dentine hypersensitivity

Tetracalcium phosphate

Calcium hydrogen phosphate

Hydroxyapatite

Octacalcium phosphate

ABSTRACT

Background: Calcium phosphates (CPs) form hydroxyapatite (HA) in physiological solutions. These are commonly used to treat dentine hypersensitivity (DH) as they mimic the mineral composition of the natural tooth.

Aim: The present study aims to characterise the apatite formation ability of a commercially available calcium phosphate Teethmate™ (TM) in physiological-like media.

Materials and methods: In this study, 4mm (D) x 6mm (L) cylindrical samples of TM were produced and immersed in tris(hydroxymethyl)aminomethane (TRIS) buffer (pH: 7.3) and artificial saliva (AS) (pH: 6.5) for up to 24 hours. This was followed by characterisation of the samples after immersion using ^{31}P magic angle - nuclear magnetic resonance spectroscopy (MAS-NMR), X-ray powder diffraction (XRD) and dentine treated with the material using scanning electron microscopy (SEM).

Results: ^{31}P MAS-NMR and XRD analyses revealed that samples immersed in TRIS buffer solution formed hydroxyapatite within approximately 6 hours of immersion. This change was observed at around 12 hours for samples soaked in AS. The pH of the immersion media increased with increasing immersion time. SEM analysis showed a transitional phase formation of structures exhibiting plate-like morphology.

Conclusion: This study shows that TM converts to HA in vitro rapidly and provides an effective option for the treatment of dentine hypersensitivity.

1. Introduction

Dentine Hypersensitivity (DH) is a significant problem caused by exposed dentinal tubules. DH is characterised by a short sharp pain in response to thermal, tactile, evaporative, osmotic or chemical stimuli [1]. Pathologic exposure of dentinal tubules results from the dissolution of enamel or denudation of the root surface. DH can develop into pulpal inflammation as a result of an invasion of dentinal tubules by oral *Streptococci* and *P. Gingivalis* and can present the clinical features of reversible pulpitis [2]. So, early diagnosis and management is critical in the prevention of DH.

There are three main theories, which explain dentinal hypersensitivity, odontoblastic transduction theory [3], neural theory [4] and hydrodynamic theory [5].

Correspondence: *Corresponding author Email Address: tomas.duminis@qmul.ac.uk.

How to cite this article: Duminis T, Shahid S. *In vitro* hydroxyapatite formation of a tetracalcium phosphate and anhydrous dicalcium phosphate based dentine desensitiser: TRIS buffer vs artificial saliva. Int J Dent Mater. 2021;3(3): 76-83. *DOI: http://dx.doi.org/10.37983/IJDM.2021.3302*

The prevalence of DH in the adult population ranges from 4 to 57% [6].

DH is treated by blocking exposed dentinal tubules with desensitising agents, such as soluble fluorides, oxalate and calcium phosphates (CPs), which reduce dentine permeability *in vitro* [6]. CPs are primarily used as bone graft substitutes and have seen significant developments in recent years, showing clinical successes and interesting biomineralisation properties [7]. CPs cements are particularly useful for the treatment of DH because in addition to reducing dentine permeability, they also chemically occlude dentinal tubules by forming hydroxyapatite (HA, $[Ca_{10}(PO_4)_6(OH)_2]$) and directly bonding to the tooth. CPs-based materials allow dentinal tubules to be sealed with a material naturally present in the tooth structure.

A commercially available desensitising agent Teethmate™ combines tetracalcium phosphate (TTCP, $[Ca_4(PO_4)_2O]$) and anhydrous dicalcium phosphate (calcium hydrogen phosphate) (DCPA, $CaHPO_4$) (Kuraray, Noritake Dental Inc., Tokyo, Japan). TTCP is also known as hilgenstockite, which was discovered by G. Hilgenstock in 1883 [8]. Once mixed with water or immersed in body fluids, these components break down to Ca^{2+} and PO_4^{3-} via a hydrolysis process resulting in nucleation and crystallisation of HA.

In vitro dentine permeability of Teethmate™ was previously studied, and it was found that this material can occlude dentinal tubules and reduce dentine permeability by 30 to 50% [9, 10]. The present study aims to understand the apatite formation ability of the material *in vitro* in artificial saliva (AS) and tris(hydroxy methyl)aminomethane (TRIS) buffer. TRIS buffer in this study provides an ion-free physiological pH medium to analyse the formation of apatite due to Ca^{2+} and PO_4^{3-} release from the material itself. Artificial saliva (AS) simulates the acidic environment in the oral cavity. These two combined give a comparison of the expected behaviour in vivo.

2. Materials and methods

2.1. Preparation of TRIS buffer solution

TRIS buffer solution was prepared by dissolving 15.090 g tris (hydroxymethyl) aminomethane (purity: ≥ 99.8%, Sigma-Aldrich, St. Louis, MO, USA) in Ca. 800 mL deionised water, adding 44.2 mL 1 M hydrochloric acid (purity: ACS reagent grade, Sigma-Aldrich, St. Louis, MO, USA), heating to 37°C overnight, adjusting the pH to 7.30 with 1M hydrochloric acid using a pH meter (Oakton, EUTECH Instruments, Malaysia) and filling to a total volume of 2000 mL with deionised water. TRIS buffer solution was kept at 37°C in an incubator.

2.2. Preparation of artificial saliva

The artificial saliva was prepared using potassium chloride (2.236 g/L), potassium dihydrogen phosphate (1.361 g/L), sodium chloride (0.759 g/L), calcium chloride dihydrate (0.441 g/L), mucin (2.200 g), and sodium azide (0.2 g) (purity: analytical grade, Sigma-Aldrich, St. Louis, MO, USA). The reagents were weighed using an electronic weighing scale and dissolved in 800 mL of deionized water. The pH was adjusted to 6.5 with potassium hydroxide.

2.3 Preparation of cement samples

To determine the apatite formation ability of the material, 4mm (D) X 6mm (L) cylinders (as given in ISO 9917-1:2007) of TM were produced (by mixing the reagents according to manufacturer's instructions and letting them set in stainless steel moulds for 1 hour at 37°C. Produced TM samples were then immersed in 10 mL of TRIS buffer and artificial saliva for 15, 30 and 45 min, 1, 3, 6, 9, 12, 15, 18, 22 and 24 hours. After each time interval, the samples were collected, blot-dried and stored in the fridge until further analysis.

2.4 Preparation of dentine discs

Caries-free teeth were embedded in impression compound and were then sectioned mid-coronally using Accutom-5 (Struers A/S, Ballerup, Denmark) to produce 1 mm dentine discs.

2.5 ^{31}P magic angle spinning – nuclear magnetic resonance spectroscopy

^{31}P MAS-NMR analyses were carried out on a Bruker Avance 600 MHz (Bruker Corporation, Billerica, MA, USA) spectrometer at a resonance frequency of 242.9 MHz in a 4 mm (outer diameter) rotor at a spinning speed of 12 kHz. Each spectrum is a sum of 16 scans. Spectra were referenced to 85% H_3PO_4 (orthophosphoric acid, 0 ppm).

2.6 X-ray powder diffraction

The samples collected after each time point were analysed using an X'Pert-PRO diffractometer (PANalytical D.V., Almelo, Netherlands). Diffraction patterns were collected from 5° to 75° 2θ. The Cu K_α X-ray frequency was λ_1=1.54059Å.

2.7 pH measurement

The pH of the immersion media was measured using a pH meter (Oakton, EUTECH Instruments, Malaysia).

2.8 Scanning electron microscopy

Samples were mounted after being dried on aluminium stubs via a self-adhesive carbon tape and were then coated using a sputter coating machine with a conductive material. Samples were analysed using an FEI Inspect F (FEI Company, Hillsboro, OR, USA).

3. Results

^{31}P MAS-NMR results presented in Figure 1 shows three spectra, t-0 (powder) one taken at 30 sec and one at 45 min after mixing with water. The peak positions are 4.8 ppm, 3.7 ppm, -0.3 ppm and -1.4 ppm with peak integrals of 1, 0.93, 0.80 and 2.51, respectively. ^{31}P MAS-NMR results shown in Figure 2(a) (TRIS buffer) and Figure 2(b) (Artificial saliva) shows how the phosphorus environment changes with increasing immersion time.

During earlier time points, the peaks are broader, which shows that the material is poorly crystalline. With increasing duration in the selected media, the peaks associated with the phosphorus environment shift upfield and narrow down, which shows that the material is becoming more crystalline because the signal is coming from more magnetically equivalent sites.

X-ray diffraction analyses of the cements soaked in TRIS buffer shown in Figure 3(a) shows how the material gradually transforms to HA. Both, TTCP and DCPA phases can be observed at one and three hours. Similar results are presented in Figure 3(b), where samples immersed in artificial saliva show X-ray scattering from TTCP, which gradually transforms to HA. DCPA at selected time points is not observed in AS. DCPA shows much higher solubility (Ksp $10^{-6.70}$) than TTCP (Ksp 10^{-38}) [11].

The results presented in Figure 4(a) and Figure 4(b) shows how the pH increases in both media with increasing immersion time and reaches a cut-off point at around 9 hours in TRIS buffer and at around 15 hours in AS where there are no further increases.

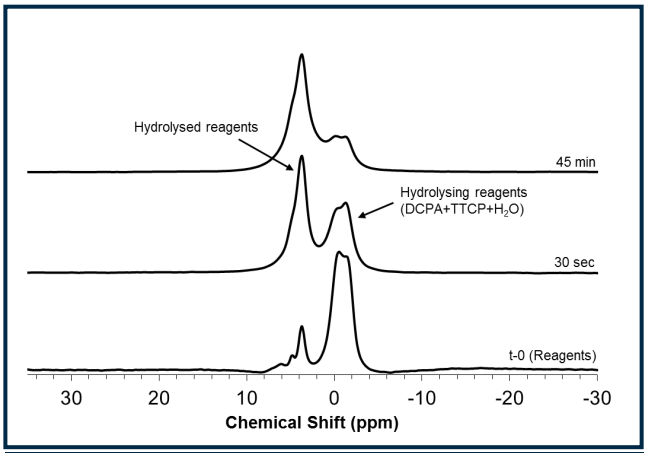

Figure 1. ^{31}P MAS-NMR spectra of the reagents mixed with water at 30 seconds and 45 minutes.

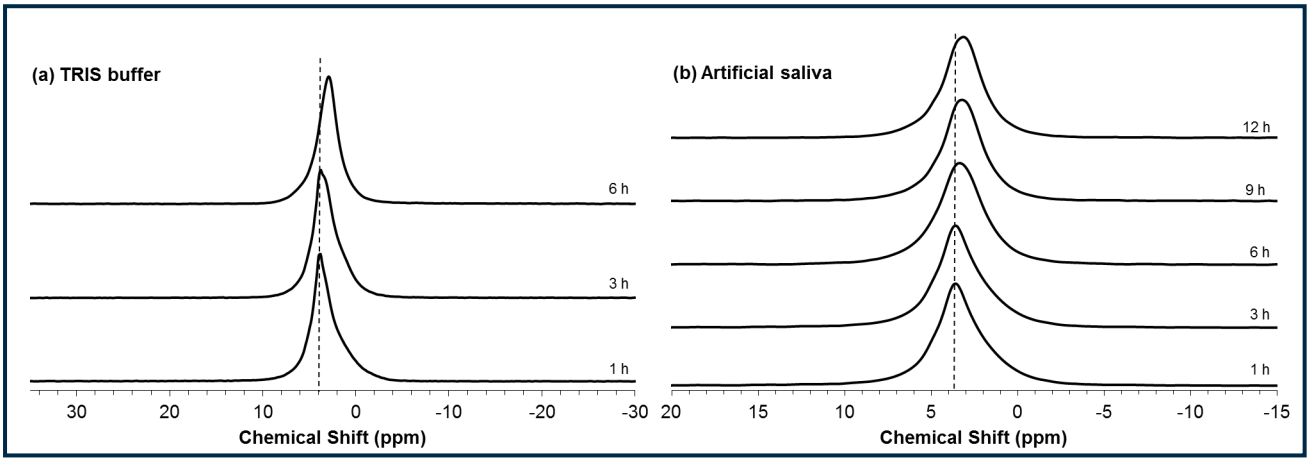

Figure 2. ^{31}P MAS-NMR spectra of cements immersed in (a) TRIS buffer solution (1, 3 and 6 hours); (b) artificial saliva (1, 3, 6, 9 and 12 hours).

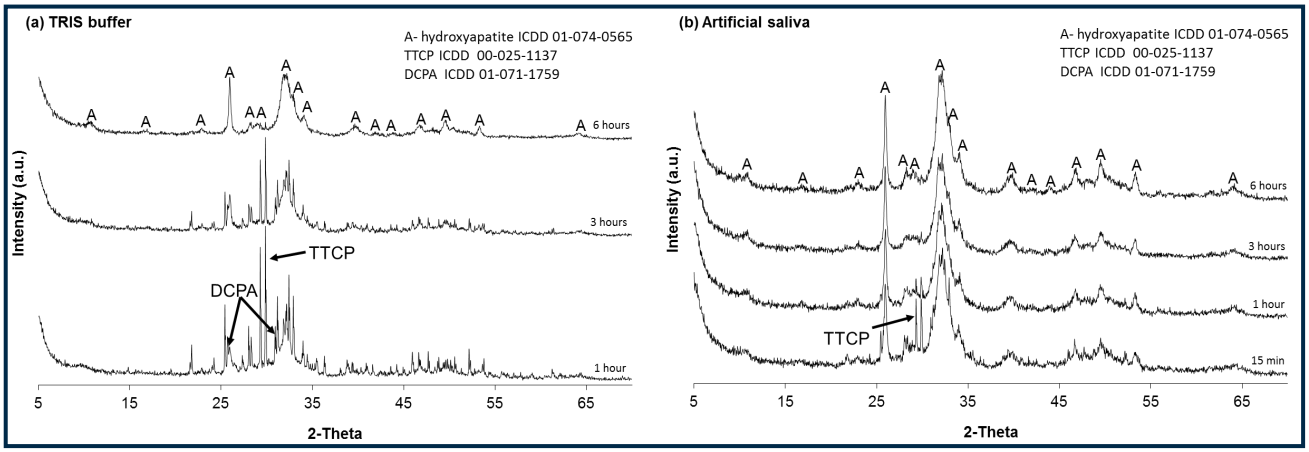

Figure 3. X-ray diffraction patterns of cements immersed in: (a) TRIS buffer solution (1, 3 and 6 hours); (b) artificial saliva (15 min, 1, 3 and 6 hours).

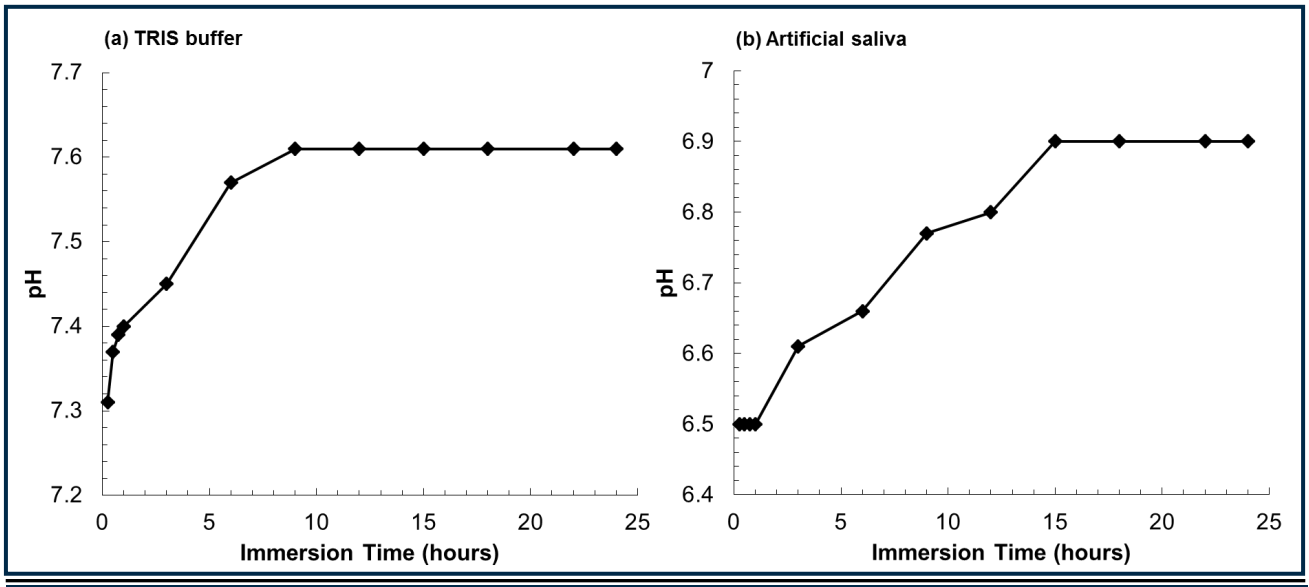

Figure 4. pH of the solutions with cement samples: (a) TRIS buffer solution; (b) artificial saliva; time: 15, 30 and 45 minutes, 1, 3, 6, 9, 12, 15, 18, 22 and 24 hours.

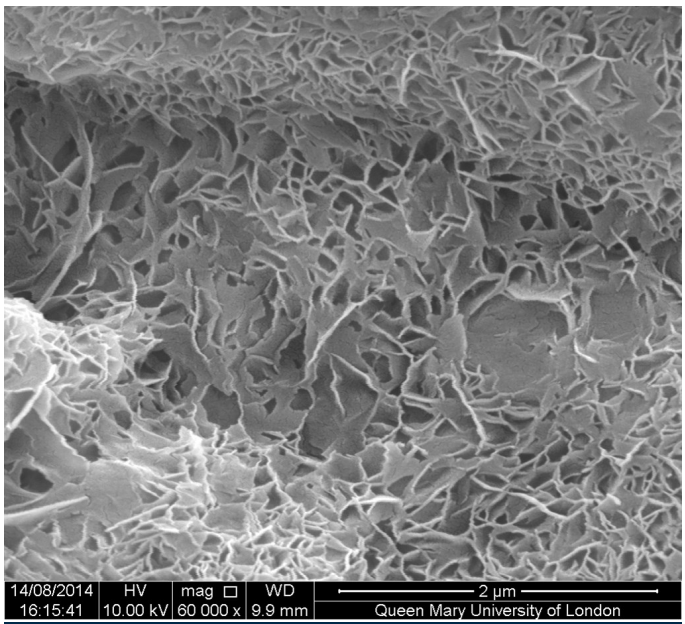

Figure 5. Scanning electron micrograph showing dentine surface treated with Teethmate™ (one hour in artificial saliva).

Scanning electron micrograph (Figure 5) of a dentine disc treated with the material and immersed in AS for one hour shows that after application, the material exhibits plate-like morphology (Figure 5).

4. Discussion

The occlusion of exposed dentinal tubules is necessary to prevent both dental pain and potential tooth infection. *In vivo* and *in vitro* synthesis of apatite is a highly complex multi-phase hydrolysis-nucleation-crystallisation process. It has been shown that the formation of HA begins with the nucleation of $Ca(HPO_4)_3^{4-}$ complexes which aggregate, take up additional calcium ions and result in the formation of $Ca_2(HPO_4)_3^{2-}$ post-nucleation aggregates, which form the basis of octacalcium phosphate (OCP, $Ca_8H_2(PO_4)_6{\cdot}5H_2O$) and HA structure [12]. OCP is considered a precursor phase in apatite formation in vivo and it is also a constituent of the human dental calculus, among other mineral phases, such as brushite ($CaHPO_4{\cdot}2H_2O$) and whitlockite (β-$Ca_3(PO_4)_2$) [13]. Using the classical crystallisation theory, it has been calculated that OCP phase formation is also a kinetically favourable process in physiological-like media [14]. The six non-equivalent sites of OCP are generally categorised into two groups, PO_4^{3-} (P1-P4) and HPO_4^{2-} (P5, P6). OCP can be described by an alternating layer structure of an apatite layer (P1-P4) and a hydrated layer (P5, P6). ^{31}P MAS-NMR peaks for pure OCP are observed at 3.7, 3.3, 2.0 and -0.2 ppm and are assigned to P1, P2/P4, P3 and P5/P6, respectively [15]. The signal at 2.0 ppm (P3) is assigned to the PO_4^{3-} at the junction of the apatitic and hydrated layers.

^{31}P MAS-NMR signals (-0.3, -1.4 ppm) from the reagents mixed with water (Figure 1) reduce in intensity and result in the development of new signals downfield (4.8, 3.7 ppm), which shows that TTCP and DCPA are reacting with water. The spectra shown in Figure 1 lack PO_4^{3-} (P3), which shows that the material does not contain pure OCP. The OCP phase may exhibit a dynamic structure when transitioning to HA with one species becoming dominant over another, which is evident from major environments observed from ^{31}P MAS-NMR (Figure 2) after immersion at around 3.7, 3.5 and 3.3 ppm preceding HA formation. These gradual changes in the chemical shift are also indicative of ongoing hydrolysis and reducing interatomic distances between phosphorus and calcium. It is known that OCP has a hydrated structure. Tseng *et al.* (2006) [16] demonstrated via ^{31}P{1H} cross-polarisation NMR experiments that water molecules enter the OCP structure during HPO_4^{2-} hydrolysis:

$$HPO_4^{2-} + OH^- = PO_4^{3-} + H_2O$$

Therefore, the signal (P3) may develop after the pH increases for the HPO_4^{2-} and OH^- to react. During further hydrolysis, the water molecules disassociate, which with the addition of calcium ions results in a HA structure.

X-ray diffraction results in Figures 3(a) and 3(b) show gradual conversion of the prepared material to HA. When immersed in TRIS buffer solution, the material formed hydroxyapatite in approximately 6 hours with a transient OCP between one and three hours. The conversion to HA phase was slower in artificial saliva where the material formed calcium-deficient HA at approximately 12 hours, with a transient OCP phase somewhere between approximately one and nine hours. The hydrolysis of the reagents was more rapid in AS than in the TRIS buffer as a result of the differences in the pH (AS: 6.5, TRIS: 7.3). The more rapid apatite formation observed in the TRIS buffer solution can be attributed to higher pH of the solution, which is a favourable factor in apatite formation [17]. This cannot be generalised and does not apply to bioactive glasses, which are used to regenerate bone, such as the Bioglass® 45S5 where at lower pH the material shows faster ion release, which facilitates a very rapid formation of HA (3 hours at pH 5.0 and 6 hours at pH 7.3) [18].

The conversion of DCPA and TTCP to HA can be observed from the reduction of intensities of the principal X-ray diffraction peaks associated with both phases. The material is engineered with an apatitic Ca to P ratio of 1.67 to facilitate stoichiometric and rapid apatite formation as described in equation 1 (Eq. 1).

$$Ca_4(PO_4)_2O + CaHPO_4 \rightarrow Ca_5(PO_4)_3(OH) \quad \text{(Eq. 1)}$$

However, it is notable that TTCP and DCPA exhibit different solubilities, so the reagents may not initially react in synergy.

The pH (Figure 4(a)) with samples in TRIS buffer reaches a cut-off point at around 9 hours with a pH of 7.6 where there are no further increases in the pH and this is the point at which the material is fully reacted and displays X-ray scattering characteristic to nanocrystalline HA. The pH of the samples immersed

in AS (Figure 4(b) also show a gradual increase until it reaches a cut-off point at 15 hours with a pH of about 6.9, which shows that the material is fully reacted with no reagents remaining. These changes in the pH occur as a result of the production of hydroxide ions during the hydrolysis of TTCP as described in equation 2.

$$Ca_4(PO_4)_2O + H_2O \rightarrow 4\ Ca^{2+} + 2\ PO_4^{3-} + 2\ OH^- \quad \text{(Eq. 2)}$$

Bioactive glasses with a high phosphate content developed by Mneimne *et al.* (2011) [19] show similar apatite formation ability in TRIS buffer (pH: 7.3) where apatite was detected at approximately 6 hours after immersion. The bioactive glasses synthesised by Mneimne *et al.* (2011) [19] showed the formation of acid-resistant fluorapatite instead of HA, which from a clinical perspective can provide longer protection under acidic conditions found in the oral cavity.

It is also notable that HA shows higher crystallinity in TRIS buffer than in AS. The ^{31}P MAS-NMR results presented in Figure 2 show HA is observed at approximately 3.0 ppm at 6 hours (assigned to HA) in TRIS buffer and at approximately 3.1 ppm (assigned to substituted calcium-deficient HA) at 12 hours in AS. Carbonate or hydrogen phosphate substitution occurs in the presence of monovalent cations. Carbonated calcium-deficient apatite is also naturally found in enamel and dentine, which shows that monovalent salivary components take part in biological hydroxyapatite formation, which results in the formation of a less crystalline calcium-deficient form of HA. AS used in the present study is saturated with monovalent cations, such as K^+ and Na^+. Tas and Aldinger (2005) [20] studied apatitic calcium phosphates formed in Na-K rich solutions and suggested that the binding of Na^+ and K^+ at the divalent Ca sites of calcium phosphates in the presence of these cations may lead to the formation of vacancies at OH^- sites which then renders the material to be more prone to CO_3^{2-} substitutions at the OH^- and PO_4^{3-} sites.

Figure 5 shows an SEM of Teethmate™ precipitate (one hour in AS) exhibiting plate-like morphology. This suggests that the material undergoes HA crystallisation via an octacalcium phosphate route. Similar morphological features were also reported by Thanatvarakorn *et al.* (2013) [21] where investigators suggested that after immersion in AS the material formed was a combination of HA with transient-formed OCP.

The average particle size of the material is 2.35µm (Supplementary Data), which is adequate to occlude dentinal tubules that have a diameter from 0.9µm (peripheral dentine) to 2.5µm (root dentine) [22].

Huang *et al.* (2016) [23] reported that strontium (Sr) can increase the potential of dentine regeneration. It was found that Sr can significantly influence the proliferation, odontogenic differentiation and mineralisation of human dental pulp stem cells (hDPSCs) *in vitro*, likely via the calcium-sensing receptor pathway. Thus, incorporation of a strontium phosphate or a strontium -containing bioactive glass additive in this material may induce a regenerative response. Keeping in view the limitations of the study, for further work it will be useful to analyse the samples using more advanced 2D CP/MAS experiments to elucidate the structure of the substituted sites.

5. Conclusion

The results presented in this study show that HA formation of a commercially available desensitising agent Teethmate™ is dependent on the composition and the pH of the immersion media. It was found that HA formation was favoured in a solution having a neutral pH as opposed to a solution with an acidic pH. The presence of monovalent cations and a low pH resulted in delayed and substituted HA formation. This may have clinical implications and may require patients to avoid eating and drinking soon after treatment.

Ethical approval and consent to participate: Caries-free extracted mandibular and maxillary molars were collected from the tooth bank with an approval from Queen Mary Research Ethics Committee QMREC 2011/99.

Conflicts of interest: Authors declared no conflicts of interest.

Financial support: None

Acknowledgements: The authors would like to thank Dr Rory Wilson for XRD analysis and Dr Nasima Kanwal for the assistance with MAS-NMR analysis (Figure 1). The authors would also like to thank Dr Alessia D'Onofrio for assistance with microscopy (Figure 5) and Dr David Gillam for the discussions about dentine hypersensitivity.

References

1. Bartold PM. Dentinal hypersensitivity: a review. Aust Dent J 2006; 51(3):212-8; quiz 276. https://doi.org/10.1111/j.1834-7819.2006.tb00431.x
2. Love RM, Jenkinson HF. Invasion of dentinal tubules by oral bacteria. Crit Rev Oral Biol Med. 2002;13 (2):171-83. https://doi.org/10.1177/154411130201300207
3. Frank RM. Attachment sites between the odontoblast process and the intradentinal nerve fibre. Arch Oral Biol. 1968; 13(7): 33 - IN39. https://doi.org/10.1016/0003-9969(68)90104-0
4. Frank RM, Steuer P. Transmission electron microscopy of the human odontoblast process in peripheral root dentine. Arch Oral Biol. 1988; 33(2):91-98. https://doi.org/10.1016/0003-9969(88)90051-9
5. Brannstrom M. The hydrodynamics of the dentine. Its possible relationship to dentinal pain. Int Dent J. 1972; 22:219-227.
6. Orchardson R, Gillam DG. Managing dentin hypersensitivity. J Am Dent Assoc. 2006; 137(7): 990-8; quiz 1028-9. https://doi.org/10.14219/jada.archive.2006.0321
7. Habraken W, Habibovic P, Epple M, Bohner M. Calcium phosphates in biomedical applications: materials for the future? Materials Today. 2016; 19(2):69-87. https://doi.org/10.1016/j.mattod.2015.10.008
8. Hilgenstock G. Eine neue Verbindung von P_2O_5 und CaO. Stahl und Eisen 1883; 3(498): 13.
9. Zhou J, Chiba A, Scheffel DL, Hebling J, Agee K, Niu LN, Tay FR, Pashley DH. Effects of a Dicalcium and Tetracalcium Phosphate-Based Desensitizer on *In Vitro* Dentin Permeability. PLoS One 2016; 11 (6):e0158400. https://doi.org/10.1371/journal.pone.0158400
10. Ishihata H, Kanehira M, Finger W, Takahashi H, Tomita M, Sasaki K. Effect of two desensitizing agents on dentin permeability *in vitro*. J Appl Oral Sci. 2017;25(1):34-41. https://doi.org/10.1590/1678-77572016-0228
11. Chow LC. Solubility of Calcium Phosphates. Monogr Oral Sci. 2001; 18:94-111. https://doi.org/10.1159/000061650
12. Habraken WJ, Tao J, Brylka LJ, Friedrich H, Bertinetti L, Schenk AS, *et al.* Ion-association complexes unite classical and non-classical theories for the biomimetic nucleation of calcium phosphate. Nat Commun. 2013; 4:1507. https://doi.org/10.1038/ncomms2490
13. Schroeder HE, Bambauer HU. Stages of calcium phosphate crystallisation during calculus formation. Arch Oral Biol. 1966; 11(1):1-14. https://doi.org/10.1016/0003-9969(66)90112-9
14. Lu X, Leng Y. Theoretical analysis of calcium phosphate precipitation in simulated body fluid. Biomater. 2005; 26(10):1097-108. https://doi.org/10.1016/j.biomaterials.2004.05.034
15. Tsai TWT, Chan JCC. Chapter 1 - Recent Progress in the Solid-State NMR Studies of Biomineralization. In Annual Reports on NMR Spectroscopy, Webb GA., Ed. Academic Press: 2011; Vol. 73, pp 1-61. https://doi.org/10.1016/B978-0-08-097074-5.00001-3
16. Tseng YH, Mou CY, Chan JCC. Solid-State NMR Study of the Transformation of Octacalcium Phosphate to Hydroxyapatite: A Mechanistic Model for Central Dark Line Formation. J Am Chem Soc. 2006; 128(21): 6909-6918. https://doi.org/10.1021/ja060336u
17. Elliott JC, Chapter 1 - General Chemistry of the Calcium Orthophosphates. In Studies in Inorganic Chemistry, Elliott JC., Ed. Elsevier: 1994; Vol. 18, pp 1-62. https://doi.org/10.1016/B978-0-444-81582-8.50006-7
18. Bingel L, Groh D, Karpukhina N, Brauer DS. Influence of dissolution medium pH on ion release and apatite formation of Bioglass® 45S5. Materials Letters. 2015; 143: 279-282. https://doi.org/10.1016/j.matlet.2014.12.124
19. Mneimne M, Hill RG, Bushby AJ, Brauer DS. High phosphate content significantly increases apatite formation of fluoride-containing bioactive glasses. Acta Biomaterialia. 2011; 7 (4): 1827-1834. https://doi.org/10.1016/j.actbio.2010.11.037
20. Tas AC, Aldinger F. Formation of apatitic calcium phosphates in a Na-K-phosphate solution of pH 7.4. J Mater Sci: Mater Med. 2005; 16(2): 167-174. https://doi.org/10.1007/s10856-005-5919-5
21. Thanatvarakorn O, Nakashima S, Sadr A, Prasansuttiporn T, Ikeda M, Tagami J. In vitro evaluation of dentinal hydraulic conductance and tubule sealing by a novel calcium-phosphate desensitizer. J Biomed Mater Res B Appl Biomater. 2013;101(2): 303-9. https://doi.org/10.1002/jbm.b.32840
22. Garberoglio R, Brännström M. Scanning electron microscopic investigation of human dentinal tubules. Arch Oral Biol 1976;21(6):355-62. https://doi.org/10.1016/S0003-9969(76)80003-9
23. Huang M, Hill RG, Rawlinson SC. Strontium (Sr) elicits odontogenic differentiation of human dental pulp stem cells (hDPSCs): A therapeutic role for Sr in dentine repair? Acta Biomater. 2016; 38: 201-11. https://doi.org/10.1016/j.actbio.2016.04.037

Supplementary Data

Particle size analysis of Teethmate™ powder component (μm)

Sample	1	2	3	STDV	Average
D[v,0.9]	8.11	8.83	11.3	1.67	9.41
D[v,0.1]	0.39	0.41	0.36	0.03	0.39
D[v,0.5]	2.34	2.33	2.38	0.03	**2.35**
D[4,3]	3.58	4.03	5.36	0.93	4.32
D[3,2]	1	1.03	0.96	0.04	1.00

The samples (*N=3*) were dispersed in deionised water and analysed using laser diffraction (Malvern 2000, Malvern Instruments, Worcestershire, UK).