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Tannic acid speeds up the setting of mineral trioxide aggregate cements and improves its surface and bulk properties

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Abstract

Hypothesis: The setting time and mechanical properties of cements are a major technical concern for a long time in civil engineering. More recently those practical problems became a major concern for biomedical applications -in bone surgery and in dentistry- in particular concerning the setting time which should be minimized. The possibility to add organic additives to interact with the different constituting ions in cements constitutes a way to modify the setting kinetics. We made the assumption that a hydrolysable polyphenol like tannic acid could modify the setting time and the physical properties of Mineral Trioxide Aggregate (MTA).

Experiments: Tannic acid is added in variable proportions to the water used to set MTA. The formation of the hybrid organic-mineral cements is investigated using a combination of structural, chemical and mechanical methods. X-ray tomography was also used to investigate the changes in porosity and pore size distribution upon incorporation of tannic acid in MTA based cements. The hydrophilicity of the cements was evaluated by measuring the permeation kinetics of small water droplets.

Findings: We found that tannic acid allowed to reduce markedly the setting time of MTA based cements. The obtained cements have an increased hydrophilicity and display excellent resistance to compression. The number of pores but not the average pore size is also affected. The possible roles of tannic acid in modifying the cement properties are discussed.

1.Introduction

The setting of concrete and of cements was and still remains a relevant technological problem during the two last millenaries. As the main control parameters are the water/cement ratio, the temperature at which the mixing is performed, the addition of additives allowing to speed up or to slow down the setting time of the cements, as well as less controlled parameters as the size distribution of the used oxide particles and the way of mixing [1]. Overall, the setting of concrete or cements for the building industry is a pretty slow process, considered to be achieved in 28 days. Fortunately, a full set of characterization methods and standards are now available to characterize, in a non-destructive manner, the time evolution of concrete and cements [2-4].

Besides their use in civil engineering, cements are also used for surgical and dental applications where they require a rapid setting and well controlled, reproducible characteristics to afford not only mechanical properties but also to control the interactions with biological tissues. Among those dental cements, Mineral Trioxide Aggregate (MTA) is a material considered as the gold standard for several endodontic treatments being a bioactive cement [5, 6]. It was used in incomplete root formation [7], the treatment of perforations and resorptions [8, 9], pulp capping [10], pulpectomy [11] and apical plug filling after apicoectomy [12]. MTA was developed and introduced in dental treatments in 1993 [13]. MTA cements are hydraulic and set in a moist medium. However, concerning their biomedical applications, these cements have a major limitation, namely their long setting time [14]. Ideally the setting should be achieved in a few tens of minutes.

Traditional MTA cements are tricalcium silicate-based materials related to Portland cement with the addition of the radiopaque bismuth oxide [5]. This bismuth oxide can be leached in time promoting discoloration of dental tissues and marginal gingiva [15]. Other MTA formulations without bismuth oxide were introduced as MTA Biorep (ITENA Clinical, Paris,

France) and MTA Repair HP (Angelus, Londrina, Brazil). These MTA cements contain calcium tungstate as a radiopaque agent, which is more biocompatible than bismuth oxide [16]. Another main difference between these two MTA cements is that the liquid of the MTA Biorep formulation contains an organic plasticizer with distilled water in order to improve the handling, during the insertion of the material into the root canal and to provide greater plasticity to the material [17, 18].

Appropriate physical and chemical properties are recommended for these dental cements such as low solubility, good sealing ability, high compressive strength, regeneration of periapical tissues, radiopacity and biocompatibility [5-7, 14-20].

The solubility of these cements in tissue fluids plays a role in the sealing quality. In addition, the dissolution of this material creates micro-leaks that lead to a loss of adhesion with dental surfaces [21]. Several studies noted that there is a relation between the solubility and the initial porosity of dental cements, thus more porous materials are more likely to have higher solubility [22, 23].

The wettability of these materials reflects their interactions with the contacting solvents and tissues. Therefore, fluid sorption testing reflects the chemical quality of the thin surface layer of these cements [24].

The good compressive strength of cements is an essential factor when it comes to coronal treatments, for example, pulp capping. However, when applied as an apical plug where there are minimal applied forces, the cement's compressive strength will be of lower importance [20, 25] than in coronal treatments. In addition to these properties, high pH values produced by these materials determines their antibacterial activity and their mineralization ability for calcium phosphates [26, 27].

All these chemical and physical properties may affect the quality of endodontic dental treatments including the root canal retrograde and orthograde treatments. Simultaneous control

of all those requirements is by far not yet achieved by recent cement formulations. Such control may be achieved by adding well suited additives to the cement.

Indeed, some additives in the composition of MTA cements were already used to modify their physicochemical properties. As typical examples, polyvinyl alcohol [28], methylcellulose [29], calcium chloride [30], calcium carbonate nanoparticles [30] and aluminium fluoride [31], rosin [32] were used as additives in MTA formulations. The aim of those additives is multifold. In order of decreasing priority, they are aimed to:

- (i) Speed up the setting process
- (ii) Reduce the dissolution of the cement
- (iii) To be able to modify the porosity and the overall hydrophilicity of the cement

All these attributes required by additives are aimed to modify the surface properties of the cements' grains which are essentially calcium silicates, calcium trisilicates and calcium oxide. It would hence be of interest to test the influence of an additive adsorbing in an almost undistinguishable manner to the surface of all these particulate materials.

Among possible candidates to fulfil such a requirement, Tannic acid (TA) is a natural polyphenolic compound of low cost which has been shown to adsorb on the surface of almost all known classes of materials [33]. TA is also largely used for industrial and biomedical applications [34, 35]. It is an antioxidant, antiangiogenic, and anti-inflammatory factor. It has been used as a stabilizer and a cross-linker for collagen, elastin and gelatin [36-39].

Several studies [40-42] have analysed and evaluated the effect of different TA concentrations for different application times as a root canal irrigant cleanser. Bedran-Russo *et al* [43] studied the collagen-dentin structure treated with TA solutions and noted that TA increases the stiffness of demineralized dentin and reduces the enzymatic degradation of collagen most probably due to hydrogen bonds between the biopolymer and TA.

However, the use of TA as an additive in cements is poorly described. Hence, in the present study, we investigate the effect of different TA concentrations on MTA Biorep in order to ameliorate its physicochemical properties, namely its setting time, its mechanical properties, its porosity and hydrophilicity. The choice of TA as an additive has been motivated by its low cost its high solubility in water and its known interactions with metallic cations among them Ca^{2+} [44] which is a major constituent of MTA. We will call these composite materials MTA@TA but their composition refereeing to the formulation step will be noted MTA $x\%$ where x is the mass fraction of MTA, the remaining mass fraction of the solid being TA (at a constant added mass of water).

2. Materials and Methods

Mineral trioxide aggregate “MTA Biorep” (Itena Clinical, Paris, France) has been modified with different weight percentages of tannic acid (TA, ref. 403040, Sigma Aldrich, Saint-Quentin-Fallavier, France). TA was used as received but stored in a closed bottle to avoid its progressive hydration, hydrolysis and oxidation. All groups of samples, called MTA@TA blends in the following, were mixed according to the manufacturer’s instructions: (120 ± 10) μL of liquid (water + plasticizer, both provided by the furnisher) for one cement capsule (24 mg of powder). The cements are called according to their composition MTA ($x\%$) where x is the mass fraction of MTA in the initial blend. For instance, MTA 75% describes a formulation in which 75 % of the solid mass is MTA and hence 25 % of the solid mass is TA. Note the sample size was different, for technical reasons, concerning each characterization technique. Therefore, we always specify the size and shape of the prepared MTA@TA samples.

2.1 Colour of the different MTA@TA samples

One sample of each cement concentration was mixed and compacted into a Teflon mould (10 mm in diameter and 3 mm in height) in order to observe the effect of the TA concentration on

the cement colour. A camera (Nikon D5300, Tokyo, Japan) with ring flash was used to evaluate the colour changes for all the tested cements.

2.2 pH measurements of the aqueous solution in contact with the cement

Immediately after mixing the cement, 10 polyethylene tubes measuring 1.6 mm in diameter and 10 mm in height [45, 46] were filled and compacted with freshly prepared samples of each cement group using a spatula and an endodontic K-file (Micro-Mega, Besançon, France). Cement residues were cleaned from the external walls. Radiographic images were taken for all the filled tubes with Vita scan (Durr Dental, Bietigheim, Germany) to verify that there is no void into the filled tubes. Each sample was immersed in a glass vial with 10 mL of distilled water (initial pH = 6.8) taken from a distilled water system Elix® Millipore (Merck Chimie SAS, Ile-de-France, France). The pH measurements were performed at (24 ± 2) °C after incubation at 37 °C for 3h, 24h, 72h and 168h. Before each pH measurement, the pH meter was calibrated with standard solutions at pH 4, 7 and 10 (Hanna Instruments, Lingolsheim, France). Before each individual pH measurement, the pH meter electrode was rinsed with distilled water for 30 s to eliminate the contamination from the previous solution.

2.3 Spectrophotometric characterization of the solutions in contact with the MTA cements

TA has been dissolved in distilled water in order to obtain a mother solution at 0.05 mg.mL⁻¹. One disc of pristine MTA (without tannic acid) was mixed and compacted into Teflon moulds as previously described with glass slides beneath (Knittel glass, Braunschweig, Germany). After the setting time (15 min), 1 mL of a TA solution was dropped onto the sample surface. After 25 min, 0.5 mL of the dropped solution and 0.5 mL of the mother solution were taken using a pipette and diluted 10 times with distilled water. Then, 1 mL of each diluted solution was analysed in the wavelength range between 200 and 700 nm using a mc² spectrophotometer (SAFAS, Monaco) in order to identify the changes undergone by TA in contact with an MTA cement. We performed this test in order to show that the colour change is due to the interaction

between TA and the MTA cement both components being either directly mixed at the onset of the setting process or during subsequent contact between the cement and a TA containing solution.

In another set of experiments, one sample of each MTA@TA composite was prepared (3 mm in diameter and 3.58 mm in height) and immersed in 10 mL of distilled water for one month. After this duration, 1 mL of each solution was taken using a pipette and diluted 10 times with distilled water. Then, 1 mL of each diluted solution was analysed in the wavelength range between 200 and 700 nm using a mc² spectrophotometer to evaluate the release of TA from the cement.

2.4 Scanning electron microscope (SEM), thermogravimetric analysis (TGA) and energy dispersive x-ray (EDX) analysis

Thermogravimetric (TGA) analysis (Netzsch) was performed on MTA and MTA 75 % samples after drying and also for the same samples immersed in water during 24 h in order to determine the water and TA weight fractions in the materials. Before TGA analysis, the samples were crushed into powder with a mortar. The temperature was increased from 25 to 700 °C under a nitrogen flow.

Concerning the samples for EDX analysis, three samples of each MTA or MTA@TA formulations were compacted using a spatula into cylindrical Teflon moulds-5 mm in diameter and 2 mm in thickness. After the setting time (15 min) as described in the manufacturer's instruction, the disks were removed from their moulds and mounted onto aluminium SEM stubs. The samples were sputter-coated with 20 nm gold-palladium (20/80 atomic % respectively) using a Hummer JR sputtering device (Technics, CA, USA). 1h later, the morphological and chemical characteristics of the MTA@TA samples were analysed using a Quanta 250 FEG scanning electron microscope (FEI Company, Eindhoven, The Netherlands)

with an acceleration voltage of the electrons of 10 kV. Five SEM micrographs were taken for each sample. Five EDX analyses were performed for each MTA@TA sample with a working distance of 10 mm and an acquisition time of 60 s. The weight percentages of Ca, C, Si and Al of the specimen surfaces of the different MTA@TA blends were determined in a semi-quantitative manner from these experiments.

2.5 Setting time of the cements as evaluated by dynamic rheometry

The storage and loss moduli of the as prepared MTA and MTA @ TA formulations were measured in a cone plate geometry with a Kinexus Ultra rheometer (Malvern, Great Britten) at a constant frequency of 1 Hz and a shear strain of 1 %. The upper plate had a diameter of 4 cm and an apex angle of 176°, the distance between the apex of the upper plate and the lower flat plate, both in stainless steel, was of 150 µm. The water, containing the desired amount of TA was mixed with the required mass of MTA on the lower plate of the rheometer, the upper plate was approached at the final separation, and the measurement started 1 min later, with a time resolution of 30s.

2.6 Compression tests

The effect of tannic acid on the mechanical characteristics of the MTA based cements has been studied. For this purpose, the samples were prepared according to the following protocol: immediately after mixing with water, different cements (MTA or MTA@TA) were placed and compacted in a Teflon mould (3 mm in inner diameter and 3.58 mm in inner height) with glass slides underneath to provide a flat surface. The dimensions and preparation methods of these samples were based on Bernardi et al [46]. Then, the moulds were transferred into a chamber hold at 37°C and a relative humidity of 95% for 6 h [47]. After that, the cylindrical cement samples were removed from their moulds and checked under 10X magnification using a Zumax microscope (Zumax medical, Suzhou New District, China) for the presence of an eventual void or a fractured edge. All damaged samples were discarded before further mechanical analysis.

Part of samples were picked to be wet. They were immersed during 24h in distilled water at 37°C.

The samples were tested through a uniaxial compression test to determine the stiffness of the cement and the maximum load before rupture. These tests were performed on the universal electromechanical tensile Instron 3345 (Norwood, MA, USA) device instrumented with a 1kN cell force (Class 0.5 following ISO 7500-1) and with a displacement sensor. The tests were performed at constant crosshead speed of 0.5 mm.min⁻¹. Deformation was calculated from the crosshead displacement. A camera (Allied Vision, Stadtroda, Germany) was used to measure the specimen dimensions to avoid any initial damage that could be caused by the standard dimensional measurement methods.

The engineer compressive stress was calculated according to the formula:

$$\sigma_c = \frac{4P}{\pi D_0^2} \quad (1)$$

where P is the recorded load during the test and D_0 is the initial sample diameter.

The engineer compressive strain was calculated using the following formula

$$\varepsilon_c = \left| \frac{\delta L}{L_0} \right| \quad (2)$$

where δL is the recorded displacement of the crosshead and L_0 is the initial length of the sample.

2.7 Water sorption tests by the MTA@TA cements

Three samples of each group were prepared and compacted in Teflon moulds (16 mm in diameter and 3 mm in height) that were glued to a glass slide and stored in hermetic boxes for 24 h. These samples were larger than those used for other characterization techniques in order to allow for the deposition of a 5 µL water drop. A contact angle measurement device (Attension Theta, Biolin Scientific, Göteborg, Sweden) was used to measure the adsorption time of a 5µL

drop of distilled water into the cement surface. A drop of distilled water was dropped via a needle, 0.7 mm in diameter, onto the surface of each sample. A movie was recorded with a horizontal camera in order to track the profile of the water drop and the absorption time of the water drop until its disappearance into the cement surface. This measurement is a qualitative indication of the cement's hydrophilicity. Static water contact angle analysis could not be performed on the MTA@TA cements owing to their high roughness and porosity.

2.8 X-ray tomography

The internal structure of the MTA based materials was inspected in 3D by means of micro-computed x-ray tomography (μ CT) (EasyTom 160 from RX Solutions, Chavanod, France). For this characterization, the as-processed material rod-identical to that used for the compression tests- was glued onto a graphite sample holder. Imaging was conducted at a voltage of 45 kV and a current of 160 μ A, using a micro-focused tube equipped with a tungsten filament. The source-to-detector distance (SDD) and the source-to-object distance (SOD) were adjusted in such a way to obtain a voxel size of around 2.1 μ m. The volume reconstruction was done with the software Xact64 (RX Solutions) after applying inherent treatments to the technique such as geometrical corrections and ring artefact attenuation. The image treatment was performed with the Avizo software that enabled to (i) de-noise the images with a median filter, (ii) segmentate the images intensity to reveal the objects of interest (pores or inclusions), (iii) remove insignificant small objects, and (iv) determine the 3D geometrical aspects of the objects (volume, equivalent diameter, density...).

3.Results and Discussion

3.1 pH changes of water in contact with the cements

The addition of Tannic acid (TA) up to 25% (w/w) of the total mass of the solid fraction, in the presence of a given volume of water, allows to produce compact cements which can be

manipulated with tweezers and by hands. However, higher weight fractions of TA do not allow to form a compact cement, most probably in relation to a reduction of the amount of available water which is used simultaneously to dissolve the highly hydrophilic TA and to set the cement. We nevertheless characterized the MTA 50 % sample by means of EDX to determine its composition but the mechanical characterization of those samples was a non-sense because of the apparent brittleness of the obtained material.

All these cements, when put in contact with water, induce a pH increase (Fig. 1) as expected for MTA cements [19] containing CaO as a basic compound. CaO undergoes hydrolysis in water to produce highly soluble calcium hydroxide at the origin of the pH increase. However, when TA is incorporated as a compound of the initial blend, the pH of the water in contact with the cement is reduced, resulting in a pH reduction close to 2 units for the MTA 75% cement compared to the MTA 100 % cement. This finding may be related to the interaction of TA with the surface of CaO as well as with the surface of almost all known materials including metals and polymers [33]. In the case of MTA this interaction may well be a complexation with the Ca^{2+} cations [44] slowing down the hydrolysis of CaO. Owing to an average pKa value of 8.5 (TA is in fact a weak polyacid with a distribution of pKa values) [50] it is unlikely that TA can exert a buffering role for solutions having a pH higher than 9 as found in Fig. 1. We hence assume that the strong pH reduction of MTA doped with TA is due to a protecting effect of TA by slowing down the hydrolysis of CaO.

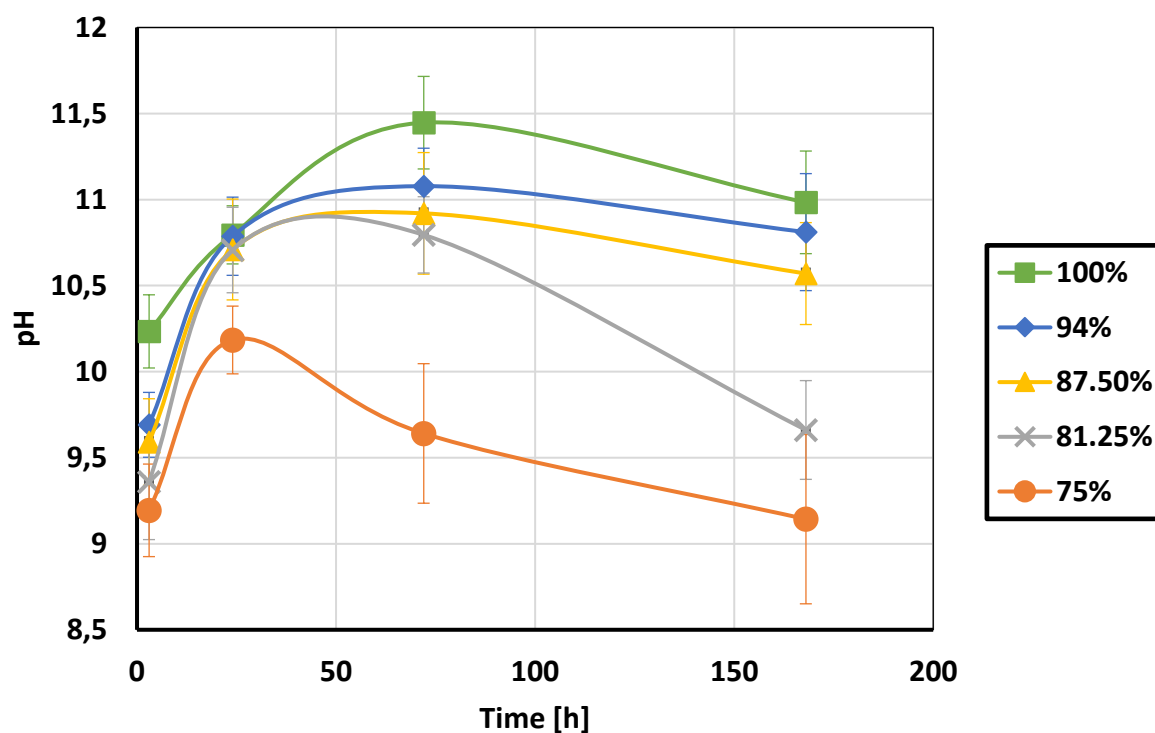


Fig.1: pH changes of water in contact with MTA and MTA@TA materials (indicated in the inset where the mass percentage corresponds to MTA) as a function of time up to 1 week. Each point corresponds to the measurement on 10 independent MTA or MTA@TA samples and the error bars correspond to \pm one standard deviation.

3.2 Colour change and composition

The MTA based cements prepared in the presence of TA undergo also a marked colour change (Fig. 2) as expected when TA is present in a high pH environment (Fig. 1) and in the presence of spontaneously dissolved oxygen acting as an oxidant.

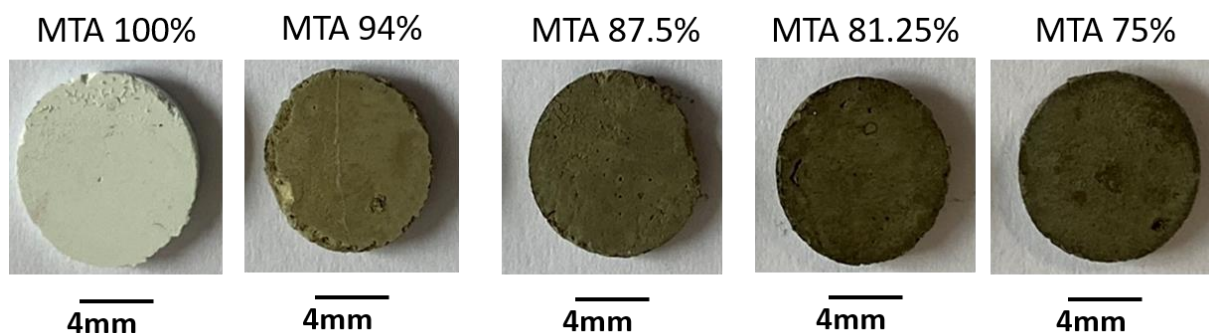


Fig. 2: visual changes of MTA and MTA@TA materials in contact with water for 24 h and dried before image capture.

This visual change in the MTA based materials is clearly due to the incorporation of TA. This colour change may be detrimental for aesthetic reasons, this is however not the case because MTA is never incorporated in the part of the teeth directly accessible to visual inspection.

The presence of TA and water was confirmed by means of TGA analysis: in the case of dry MTA and hydrated cements: the percentage of mass loss was of $(15.0 \pm 0.5) \%$ in the temperature range between 25 and 700°C whereas the mass loss amounted to $(35.0 \pm 0.5) \%$ in case of both the dry and wetted MTA 75 % samples, indicating the presence of additional thermosensitive molecules, namely TA. In addition, a semiquantitative quantification of the Ca and C content was performed by EDX analysis and confirmed, as expected, an almost linear decrease in the Ca content and an almost constant linear increase in the C content upon incorporation of a higher mass fraction of TA in the initial blend (Fig. 1 in the Supplementary material file).

These results mean that a given mass of MTA cement should have a given capacity to interact with TA and to modify its properties.

3.3 Setting time and morphology

Among the properties modified upon the incorporation of TA, are the setting time, as investigated by shear rheology and the morphology as investigated by SEM (Fig. 3). It appears that the presence of TA in the formulation speeds up the setting time of MTA cements. Indeed, in the absence of the polyphenol, the setting kinetics displays a lag phase typical of a nucleation- followed by crystal growth- process [50]. However, in the presence of 12.5 % weight percent of TA (the MTA 87.5% samples) and 25 % of TA (the MTA 75 % samples), the onset of cement setting is shorter than the time required to start the measurement (2 min. after mixing of the different compounds) and a plateau level in the storage modulus is rapidly reached without the observation of a lag phase (Fig. 3A). This acceleration of the cement setting is of great interest in dental applications of MTA cements as highlighted in the Introduction and was the major goal of this investigation.

The morphology of the cement after 1 h of setting is also dependent on the weight fraction of TA used in the initial blend (Fig. 3B) with a more granular morphology and an apparent, but hard to quantify, reduction in the average grain size (Fig. 3B). In fact, the reduction in grain size when the mass fraction of TA increases may significantly affect the mechanical properties of the cement. A shorter setting time and a change of the grain morphology are strong indications that the addition of TA acts as a surface modifier in the MTA cement.

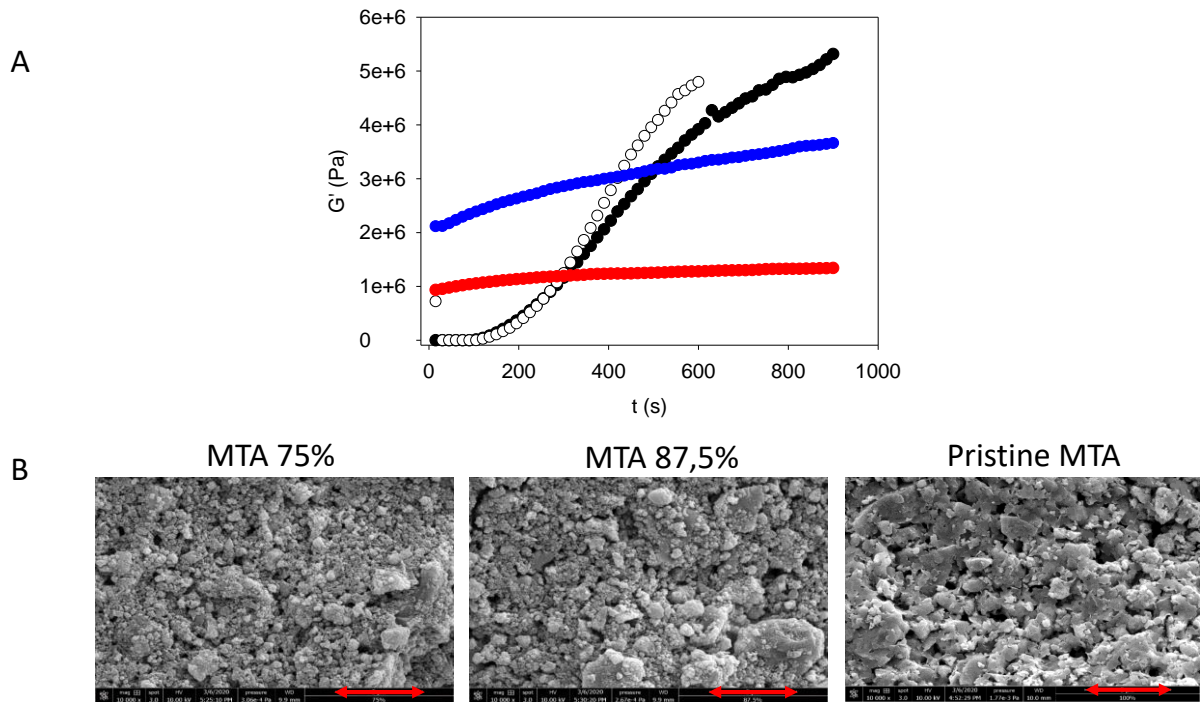


Fig.3: A: Setting kinetics of the pristine MTA (\circ , \bullet - 2 independent experiments), of the MTA 87.5% (\bullet) and of the MTA 75 % (\bullet) samples as measured by rheology.

B: Morphology of the MTA 75 %, the MTA 87.5 % and of the MTA 100 % sample after 1 h of setting. The scale bar (red double headed arrow) corresponds to 5 μ m in all images.

3.4 Mechanical properties of the cements in the dry and wet states

The compressive stiffness and the maximum compressive stress, measured after a setting time of 6 h (at 37°C) are significantly affected by the presence of an increasing amount of TA. For dried samples, we can distinguish two subgroups (Fig. 4): the first one is constituted from the pristine MTA and MTA 94%, the second one from the MTA 87.5%, MTA 81.25% and MTA 75%, hence the TA rich samples. We observe an increased dispersion in the compressive properties in the second subgroup. In fact, this dispersion can be related to the action of TA on

the setting of the cement (Fig. 3A) and to the variation of the grain size (Fig. 3B). However, even if the data are more dispersed for TA contents higher than 12.5 % in the initial formulation, compared to the data acquired for lower TA contents, the compressive modulus and maximal stress are significantly higher. Interestingly, there is no significant difference in the compressive properties for the MTA 87.5%, the MTA 81.25 % and the MTA 75 % samples for which an apparent plateau in the compressive properties seems to be reached. In our opinion the higher dispersion of the compression data at higher compositions in TA originate from the presence of a higher number of defects in the composite material in which TA may not only cover the surface of the grains but also be present as clusters. Nevertheless, when the content of TA added in the blend increases, the compressive properties markedly increase as long as the composite cement is stored in the dry state. This suggests that TA present on the surface of grains (it adsorbs on the surface of all known materials, [33]) and as clusters in the porous volume (influenced by its own presence) acts as binder. Indeed, on the sole basis of the composition of the blend (our EDX data give the real, approximate, composition as a function of the provided mass fraction of TA during the preparation of the blend, Fig. 1 in the Supplementary material), one would expect a progressive reduction in the compressive properties upon a higher incorporation of the soft organic material. This is obviously not the case, in the dry state (Fig. 4).

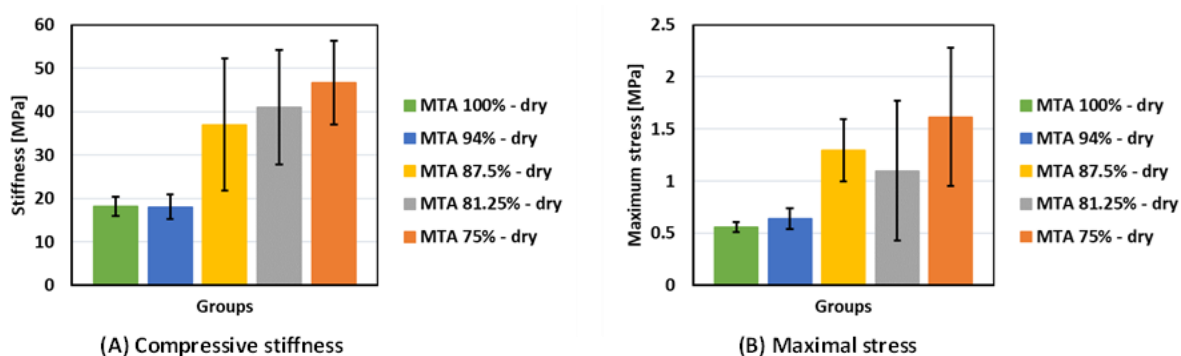


Fig. 4: Evolution of the mechanical properties of the dry MTA@TA cements ($n = 6$). A): Compressive stiffness and B): maximal stress at rupture.

The increase in mechanical properties may also be explained by the fact that the addition of TA decreases the grain size (Fig. 3B) and also by a possible chemical role played by TA adsorbing on the surface [48] of the particles and creating bonds—mostly hydrogen bonds—between them.

In opposition with the results obtained in the dry state, when the MTA based samples are immersed in water for 24 hours, the addition of TA has a negative effect on their mechanical properties (Fig. 5). On the other hand, in the absence of TA, the compressive stiffness and the maximal compressive strength increases upon hydration (Fig. 5). This is related to the well-known slow setting process of pristine MTA [14]: this cement continues to harden in the presence of water. In contrast to this finding for MTA, in the presence of TA, namely for the MTA 87.5%, MTA 81.25 % and the MTA 75% samples, after immersion, we observe a significant decrease in the compressive stiffness and in the maximum compressive stress. This decrease of the compressive mechanical properties can be explained by the fact that TA interacts with water and with the surface of the particles present in the cement. This brittleness is even more perceptible as the weight percent of TA is high.

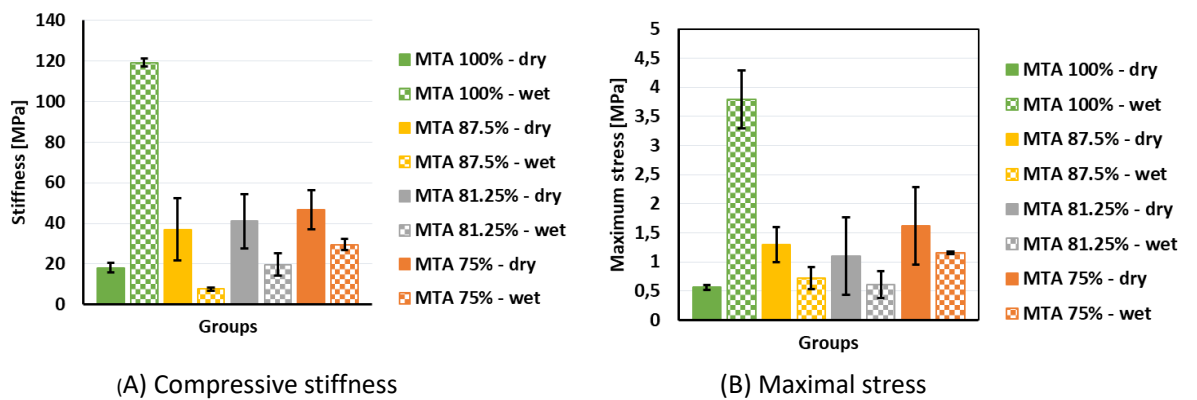


Fig. 5: Evolution of mechanical properties of wet MTA@TA cements (n=6) compared to the dry ones.

3.5 Porosity and pore size distribution investigated by X-ray tomography

In order to explain the differences in the compressive behaviour of the MTA and MTA 75% cements in the dry versus the hydrated state, we have also to consider the influence of TA on the microstructure of the cements, recalling that we suspect part of TA to be partially incorporated as clusters between the MTA grains, thus creating some additional porosity. To that aim, we performed some X-Ray tomography experiments in the case of the two extreme compositions, namely MTA 100% and MTA 75%. We decided to use X-ray tomography instead of conventional porosity measurement techniques [51] not only to avoid perturbation of the pore structure upon solvent incorporation or evaporation but also to have visual access to the pore distribution and connectivity.

When comparing the MTA 100% with the MTA 75 % in the dry state, no major differences do appear concerning the pore size (Fig. 6A and Fig. 6B, quantitative data in Table 1). Hence, we assume that the increase in compressive strength upon incorporation of TA is due to the adsorption of TA on the cement components during the setting process leading to an increased cohesion between the TA decorated particles as well as to an incorporation of part of the TA between the MTA grains. This assumption is compatible with the universal coating ability of TA [33]. However, when comparing the wet samples, it appears that the initial presence of TA induces a marked increase in connectivity (Fig 6D versus Fig. 6B) of the pores after hydration even if the MTA 100 % sample also undergoes some evolution in its pore size and pore size distribution (Table 1). We have also shown that some oxidized TA is released in water from the MTA@TA samples (Fig. 2 in the Supplementary Material file), this may be at the origin of the increased pore connectivity in this case. Such an increased pore connectivity could be at the origin of the appearance of more cracks during the compressive test and hence of a reduced

compressive resistance. In addition, TA is still strongly adsorbed on the particles surfaces and could play the role of a lubricant also influencing the mechanical properties. The presence of TA should also influence the interaction of an as prepared cement with externally added water.

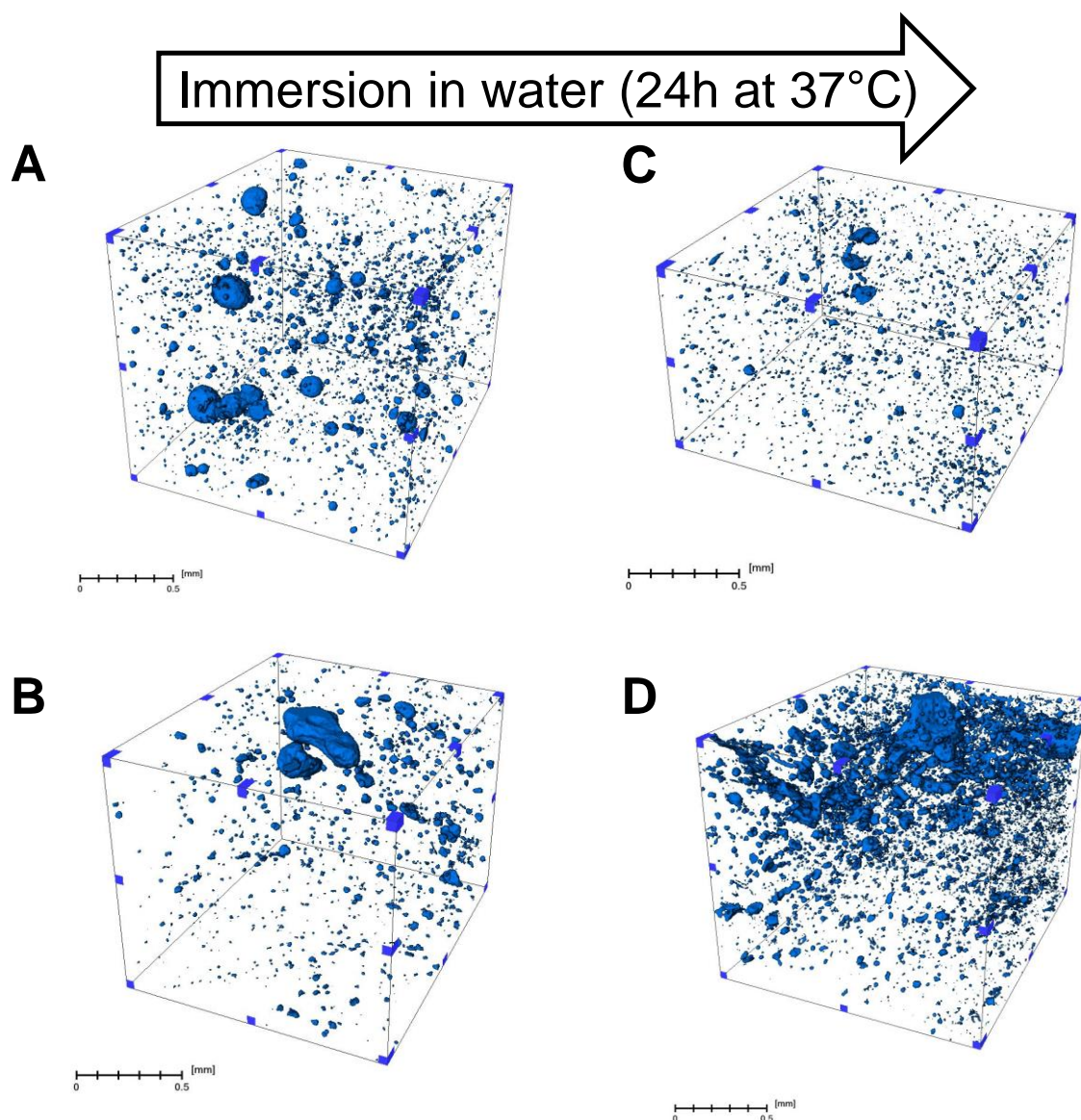


Fig. 6: Volume rendering of the segmented pores in: (A) MTA 100%, (B) MTA 75% dry samples; and (C) MTA 100%, (D) MTA 75% immersed samples in water for 24h at 37°C, obtained by X-ray tomography analysis. The scale bar corresponds to 0.5 mm in all cases.

Composite	Pore size (mm)	Density volume (%)	Number/mm³
MTA 100% (dry)	0.013	0.70	1303
MTA 75% (dry)	0.017	2.83	496
MTA 100% (wet)	0.012	2.96	1745
MTA 75% (wet)	0.013	1.7	2858

Table 1: Quantitative results from the X-ray tomography experiments.

3.6 Water sorption capacity of the cements

As another marked influence of TA on the cement properties is the increased hydrophilicity, quantified as the time required, to totally engulf a 5 μ L droplet of distilled water (Fig. 7 and Table 2).

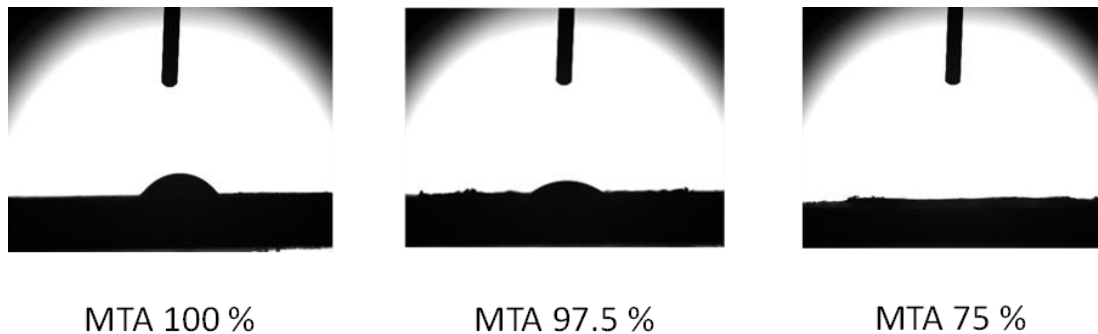


Fig. 7: Visual aspect of a water droplet (5 μ L in initial volume) deposited onto different MTA@TA cement surfaces, 5 s after its deposition. Before these experiments, the cements were kept in an ambient atmosphere for 24h after mixing the different components.

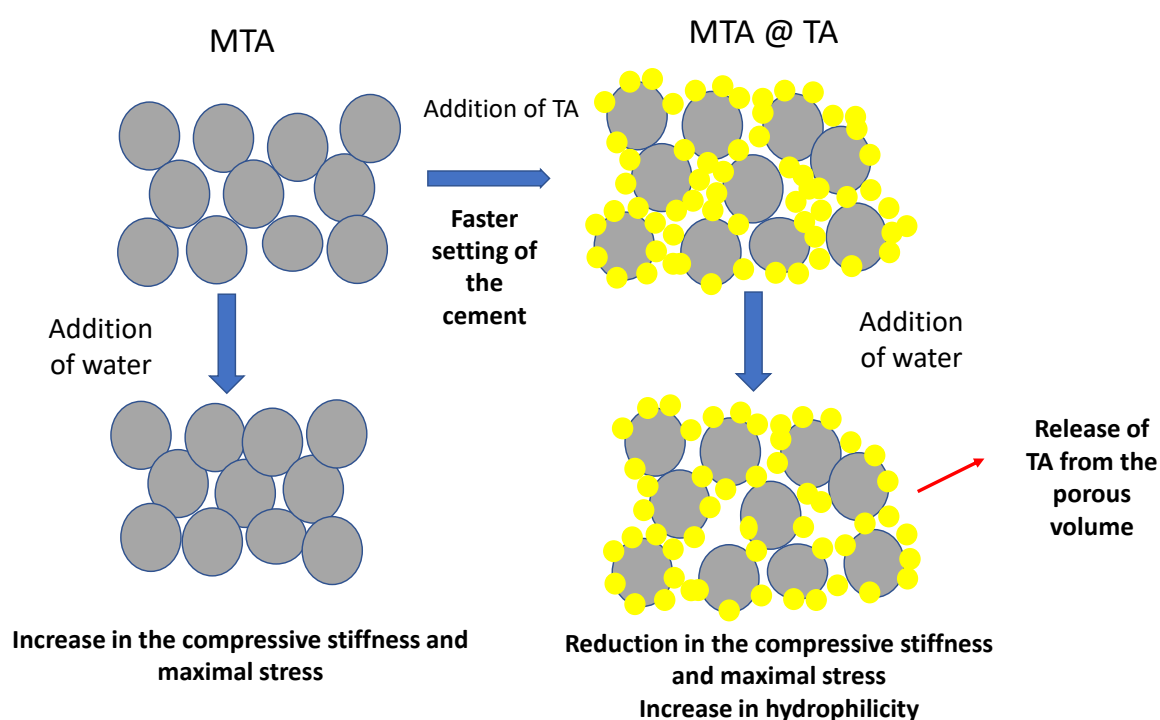
Sample	t (s)
MTA 100%	45 ± 5
MTA 94 %	30 ± 3
MTA 87.5 %	13 ± 2
MTA 81.25 %	9 ± 1
MTA 75 %	0.5 ± 0.2

Table 2: Evolution of the time required for the MTA@TA cements to totally engulf a 5 μ L water droplet.

This increased hydrophilicity of the MTA@TA cements with the initial content of TA is obviously correlated with an increase in the TA content, a highly water-soluble molecule, but may also be correlated to a change in pore connectivity of the composite cement (Fig. 6).

Taking the results from the compressive tests, from the X-ray tomography data and from the water permeation data as a function of the initial content in TA into account, we propose a hypothetical model describing the role of TA in the structure of the composite MTA based cements (Scheme 1). TA may play a dual role, depositing on the surface of the grains (calcium oxide, calcium silicates and trisilicates) and incorporating between the grains to modify the porosity of the composite. The coverage of the grains by a polyphenol based layer is at the origin of a markedly lower pH increase when the cement is put in water (Fig. 1) and could also explain the faster setting kinetics of the cement (Fig. 3 A). The mutual presence of TA on the surface of the grains and in the pores explains the increase in the compressive stiffness and maximal compressive stress in the dry state, because TA molecules form strong intramolecular hydrogen bonds and hydrogen bonds/ionic interactions with the surface of the grains. However, when the composite cements are put in the water, part of the TA present, is released in the

solution (Fig. 2 in the Supplementary material section) which produces a weakening of the composite cements (Fig. 5). Contrarily to the pristine cement put in the water, no hardening is observed because there remains some TA absorbed on the surface of the constituting grains freezing their coalescence and hindering further positive evolution. Even if the mechanical properties of the MTA@TA composites are significantly reduced in the wet state compared to pure MTA cements, this reduction is acceptable for dental applications and is largely compensated by a faster setting process (Fig. 3A) which is a major requirement for clinical use.



Scheme 1: Schematic representation of the influence of TA on the structure and the resulting properties (in bold characters) of the MTA@TA composites. This model relies on the presence of two populations in TA: adsorbed on the surface of the mineral grains and present in the porous volume of the cement which is by itself influenced by the addition of TA in increasing amounts.

4. Conclusions

The addition of TA to MTA cements decreases markedly the setting time and the grain size of the obtained cement and increases the hydrophilicity of the composite materials. All these findings give strong indications that TA modifies the surface of the cement's grains. Concerning the mechanical properties under compression, the presence of TA increases the compression modulus and maximal load before rupture in the dry state, but owing the high hydrophilicity of the polyphenol and some leaching in water, its presence in the composite has an opposite effect in the presence of water. However, the reduction of the maximal compressive stress by a factor of about 2 of the MTA@TA composites with respect to the hydrated MTA 100% cement is not detrimental for its use as an apical plug where there are minimal applied forces [20, 25]. The simultaneous increase in hydrophilicity and in brittleness in the presence of water for the TA containing cements may be explained by a dual role played by the selected organic additive: part of it is incorporated in the pores between the particles and parts of it adsorbs on the surface of the different present particles playing the role of a binder and a lubricant. The TA filling part of the pores is partially or totally released in the presence of water increasing the pore density (Table 1) and hence the pore connectivity (Fig. 6D versus Fig. 6B) and also the probability of crack propagation.

As an additional -central- question is the possibility that TA not only adsorbs on the surface of the constituting particles and fills part of the pores but may also be incorporated in the obtained phases with a possible phase change. The occurrence of such a phenomenon, which we will investigate by means of X-ray diffraction, cannot be excluded on the basis of the known interaction between TA and Ca^{2+} cations.

In future investigations, we will test the influence of other polyphenols in the setting of dental cements which will allow to build a structure-property map for polyphenols as additives in biomedical cements. We will also study the influence of this kind of water-soluble molecules

on the setting of more simple materials like plaster those components will not induce some pH increase in contact with water allowing hence to avoid the polyphenol oxidation. In such a composite TA will only act through complexation with Ca^{2+} cations. The oxidation of TA in the presence of MTA, inducing some marked darkening of the cement (Fig. 2), constitutes the only apparent drawback of our approach. However, MTA is never incorporated in the part of the teeth directly accessible to visual inspection and may not modify their aesthetic appearance. In addition, we cannot exclude that the TA modified cements display increased adhesion with resins and the dental tissues.

CRedit authorship contribution statement

Naji Kharouf: investigation and methodology

Jihed Zghal and Hamdi Jmal: investigation

Frédéric Addiego: investigation and validation

Youssef Haikel: validation

Manon Gabelout: resources and validation

Nadia Bahlouli: validation and formal analysis

Vincent Ball: conceptualization and validation

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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