SEM Analysis of Composites with TCP/HA/Chitosan/Poly (Methylmethacrilate)

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Modified cement composites were prepared by dispersing commercially available PMMA powders and chitosan/ tricalcium phosphate (TCP) or chitosan / hydroxyapatite (HA) fillers into a PMMA matrix. SEM and EDX were used to determine the compounds and the morphology of the composite. The characteristics of these materials indicate that the addition of chitosan/TCP and chitosan /HA as a constituent into the PMMA cement significantly decreases the curing peak temperature. Furthermore, the setting time increases from 4 min to 7 min, as compared to the PMMA cement. These changes could be beneficial for the handling of the bone cement paste and causing less damage to the surrounding tissues.

Keywords: PMMA, tricalcium phosphate, hydroxyapatite (HA), SEM and EDX

Inorganic bone cements such as calcium phosphate cements have been widely applied in orthopaedic and dental fields because of their self-setting ability, development of high-strength bone cement with bioactivity and biodegradability. Tricalcium silicate/magnesium phosphate composite bone cement was prepared having compressive strength up to 87 MPa, adjusted setting time (between 3 minute and 29 minute depending on the variation of composition) [1].

Another inorganic bone cement is based on calcium phosphate, which have been attracting great attention due to their excellent biological behaviour: biocompatibility, bioactivity and osteoconductivity. The real limitation of calcium phosphate cements are their low fracture toughness and poor mechanical reliability [2].

The main advantages of calcium phosphate cements are that they can be injected and have the ability to harden in vivo at body temperature [3,4].

The characteristics of being injectable and of hardening in vivo can also be found in acrylic bone cements (based on PMMA); however the hardening process of PMMA is highly exothermic, causing necrosis of the surrounding tissue. In contrast, the hardening of calcium phosphate cements is only slightly exothermic, which is important for biomedical applications [5,6].

Hydroxyapatite (HAP, $Ca_{10}(PO_4)_6(OH)_2$) is the main inorganic compound of natural bone and has been studied extensively for medical applications due to their excellent biocompatibility, bioactivity and osteoconductivity. HA powders based materials, used for the treatment of bone disease have the disadvantage of facile migration from the implanted sites. Polymeric nanocomposites exhibited improved properties, such as modulus, strength and stiffness [7]. Incorporation of HA (the mineral component of bone) with chitosan could improve the bioactivity and the bone bonding ability of the HA/chitosan composites. Such nano-hydroxyapatite/chitosan composite material prepared via in situ hybridization route, consist of homogeneous aggregations around 40-100 nm, in which many HA nanocrystals align along the chitosan molecule [8].

In our previous studies we studied the influence of hydroxyapatite [9], tricalcium phosphate [10], nano-fluoro-hydroxyapatite [11] of graphene [12] on the properties of dental composites.

Experimental part

Materials and method

Experimental biomaterials based on biocompatible polymers (polymethylmethacrylate), degradable materials (chitosan CH) and inorganic analogous components (TCP and HA) were studied. β-tricalcium phosphate [(TCP); ($Ca_3(PO_4)_2$)] was prepared by co-precipitation of calcium hydroxide ($Ca(OH)_2$) and phosphoric acid (H_3PO_4)). The process comprises in adding calcium to phosphoric acid, during continuous agitation, while regulating the *p*H and temperature. The obtained slurry is quickly dried. The TCP which is formed is rapidly dissolved in a phosphoric acidic aqueous solution. HA slurry was synthesized by a chemical precipitation method from aqueous solution of the reactants according to the literature [13], in our laboratory, and these ultrafine nano-powders were further obtained by freeze-dried procedures. Chitosan with the acetylation degree of 93.2% and molecular weight of 600 Da (Sigma-Aldrich), in the form of solid flakes of 1-8 mm, white to yellowish colour, was added in the TCP gel in 40% weight, and respectively 40% HA, was synthesized using sonication stirrer. After sonication processes, the solution was allowed to settle for 12 h at room temperature. The gel obtained was lyophilized. TCP/Chitosan/PMMA cement and HA/ Chitosam/PMMA were prepared by interpenetrating bulk polymerization of methyl methacrylate (MMA) monomer in structures of TCP-CH and HA-CH respectively. For the preparation of samples, weighed amounts of PMMA powders, tricalcium phosphate or hydroxyapatite respectively and chitosan, were mixed in a mixer mill in the presence of a peroxide initiation activator. The liquid component of the cement is made of methyl methacrylate and the polymerization activator (N,N- dimethyl-p-toluide (Merck)). Crystalline phase and microstructure of the cement with TCP/CH/PMMA and HA/CH/PMMA were

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characterized by scanning electron microscopy (SEM; FEI Company). To draw solid conclusions about the influence of the particles size, form and uniform mixing on the chemical process we acquired PMMA sorted according to granulometric size.

Results and discussions

EDX maps for sample TCP/CH/PMMA is presented in figure 1. Carbon map described the distribution of carbon both in the PPMA spheres and in the matrix form by MMA polymerization and chitosan. One can see a higher concentration of carbon into the PMMA spheres. Oxygen map revealed the uniform distribution of oxygen both in the PMMA spheres and in the matrix. Phosphorus can be observed mainly into the particles outside PMMA spheres, but the distribution suggests that the PMMA spheres are covered with a thin layer of inorganic phase. Ca distribution is similar to phosphorus distribution suggesting that the inorganic phase is highly homogenous distributed into the PMMA matrix and chitosan.

The EDX data showed that the composites contained C, Ca, P and O as expected but no N were detected which is contained by chitosan in smaller amount compared with C and O. Ca and P were thought to be originated from hydroxyapatite and TCP, O from both of the components and C from chitosan and PMMA.

The SEM images showed that the composites have a homogeneous and porous microstructure.Figure 2 presents SEM micrographs of HA/CH/PMMA and TCP/CH/PMMA samples. A uniform distribution of PMMA spheres into the matrix can be observed. The deposition of inorganic particle at the surface of the PMMA spheres is highly evident in figure 2a. SEM micrographs confirm also the uniform distribution of inorganic phase into the organic matrix. The inorganic phase seems to be form from very fine particles in both samples.

In figure 2b can be observed that PMMA spheres tend to form chains probable due to the presence of chitosan. One can also observed that the inorganic phase embedded into PMMA and chitosan adhere better to PMMA spheres in the case of HA/CH/PMMA than in the case of TCP/CH/PMMA.

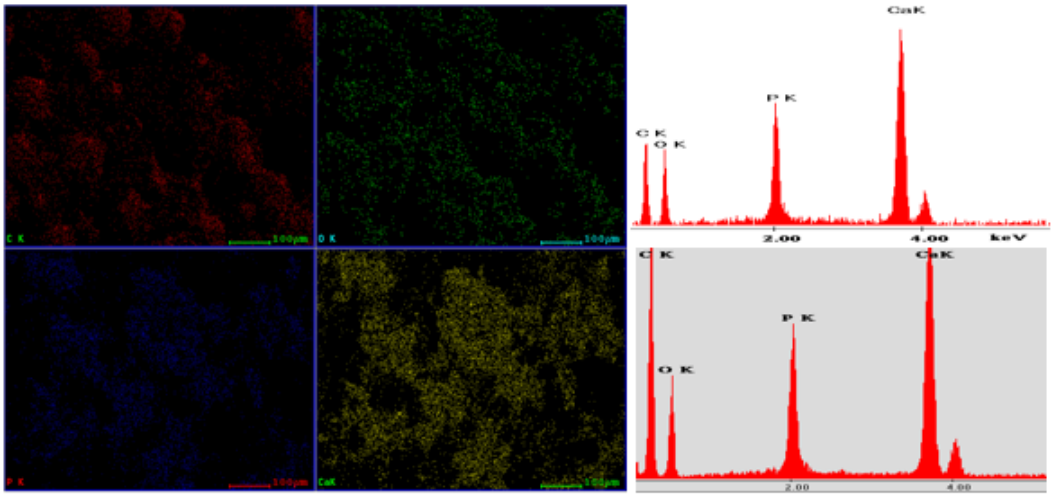
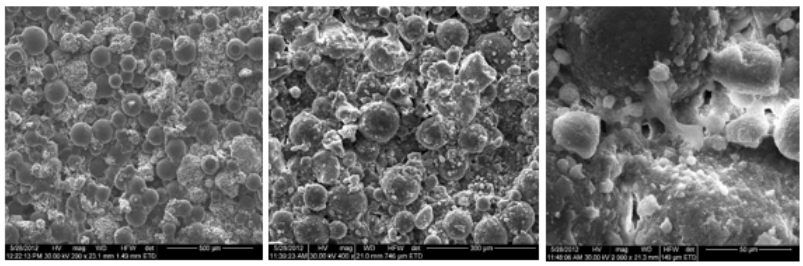
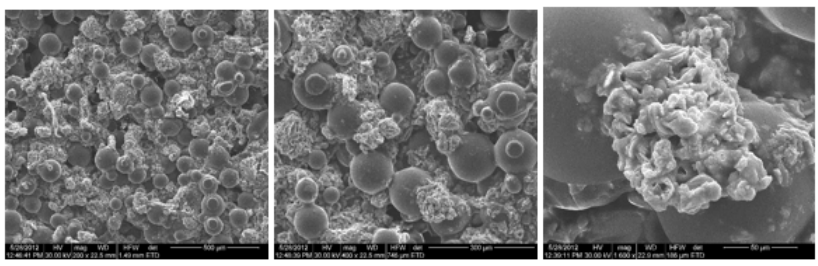


Fig. 1. EDX maps (a) for carbon, oxygen, phosphorus and calcium for sample TCP/CH/PMMA and chart (b) for sample TCP/CH/PMMA-bottom and HA/CH/PMMA-top



500 µm, mag: 200; 300 µm, mag: 400; 50 µm, mag: 2000

a. HA/CH/PMMA



500 µm, mag: 200; 300 µm, mag: 400; 50 µm, mag: 2000

b. TCP/CH/PMMA

Fig. 2. SEM micrographs for HA/CH/PMMA (a) and TCP/CH/PMMA (b)

Further investigations are needed in order to establish the influence of the interactions between PMMA spheres and the other compounds of the materials.

The curing peak temperature significantly decreases in the case of HA/CH/PMMA and TCP/CH/PMMA comparing to other systems without chitosan/TCP or chitosan /HA causing less damage to the surrounding tissues.

The setting time increases from 4 min to 7 min, as compared to the PMMA cement. These changes could be beneficial for the handling of the bone cement paste.

Roeder et al. [14] studied the effects of particle size and shape, and found that smaller particle size and larger aspect ratio increased the composite mechanical properties. Because there are few papers that study about addition of hydroxyapatite and TCP in PMMA, this study tries to fill this gap. If the proportion of the HA and TCP particles increases, this could lead to non-homogeneous distribution and, therefore, aggregation of particles may occur. This may cause phase segregation and non-homogeneity in the structure and poor adhesion to the matrix. Hydroxyapatite and TCP particles were dispersed uniformly and were embedded in the chitosan matrix. The study showed the feasibility of freeze drying method which conducted to this three dimensional porous network structure.

Conclusions

Two systems of composite materials containing chitosan, PMMA, hydroxyapatite or tricalcium phosphate have been obtained. SEM micrographs and EDX, revealed the formation of a composite materials containing PMMA spheres, chitosan and the inorganic phase (TCP or HA) embedded into a phase containing PMMA, resulted from the polymerization of MMA.

The curing peak temperature significantly decreases in the case of HA/CH/PMMA and TCP/CH/PMMA comparing to other systems without chitosan/TCP or chitosan /HA. The setting time increases from 4 to 7 min , as compared to the PMMA cement.

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