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TTCP-DCPA Based Calcium Phosphate Cements Containing Hybrid Microparticles as Drug Carriers

Hernán E. Romeo[1, a], María A. Fanovich[1, b]

[1]Institute of Materials Science and Technology (INTEMA), University of Mar del Plata and National Research Council (CONICET), Juan B. Justo 4302, 7600, Mar del Plata, Argentina.

[a]hromeo@fi.mdp.edu.ar, [b]mafanovi@fi.mdp.edu.ar

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Abstract. In recent years, considerable attention has been focused on the development of new composite materials for application as drug delivery systems. In this field, calcium phosphate cements (CPCs) are often employed as support to delivery of drugs, but their behavior has some drawback related to the so-called burst effect. The aim of this work was to develop new CPCs formulations from synthesized tetracalcium phosphate (TTCP), dicalcium phosphate anhydrous TTCP and drug-containing hybrid microparticles (DCHM). The main function of these DCHM is providing nuclei of high concentration of drugs into the CPCs. The DCHM were synthesized via the sol-gel method from a bridged precursor of the type $(H_3CO)_3$ – Bridge – $(OCH_3)_3$ and aspirin (AS) as model drug. The inorganic polycondensation reached 89.5 % as calculated by ^{29}Si NMR. The analysis by small angle X-ray scattering (SAXS) reveled a short range structural ordering in the DCHM at molecular level. Effective incorporation of AS inside the microspheres was detected by FTIR spectroscopy. In vitro tests of DCHM according to ISO 10993-5 revealed non-cytotoxic behavior. Four CPCs formulations containing 0, 1, 5 and 10 wt % of DCHM, were evaluated. The presence of DCHM did not modify neither the degree of conversion to low-crystallinity HA nor the measured setting times of the CPCs, however, the amount of incorporated microparticles considerably affected the degree of porosity (macropores of 200 μm) and interconnectivity of the cement matrix.

Introduction

Brown and Chow [1] developed a calcium phosphate cement (CPC) from tetracalcium phosphate ($Ca_4(PO_4)_2O$, TTCP) and dicalcium phosphate anhydrous ($CaHPO_4$, DCPA). These components react in an aqueous medium to form, as final product, hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$, HA) the main mineral component present in teeth and bones. Different techniques revealed that the HA formed from these CPCs has a similar morphology as that present in teeth and bones. In addition, *in vivo* studies on these CPCs showed that after implantation, the cements are gradually resorbed and replaced for new bone tissue.

On the base of their properties, the CPCs pastes can easily fill osseous cavities of any geometry, which leads to an optimal tissue-biomaterial contact [2]. In the last years, many efforts were made oriented to the development of new composite materials as support to delivery of drugs [3]. In this field, the CPCs were employed as drug carriers, but their behavior has some drawback related to the so-called burst effect. In order to improve these inconvenient, many investigations are currently in progress [4].

The aim of this work was to develop new CPCs formulations from synthesized TTCP and commercial DCPA incorporating aspirin-containing hybrid microparticles (DCHM). The DCHM were synthesized from a bridged silsesquioxane precursor via ultrasonic irradiation-assisted sol-gel method. The main function of these DCHM is providing to the CPCs of nuclei of high concentration of drugs which could be incorporated to the cement system as the setting process occurs. The morphology of the synthesized microparticles, their cytotoxicity and the influence of their incorporation into the cement matrix on the setting times and final porosity of the hardened systems, were analyzed.

Materials and Methods

Synthesis of Drug-Containing Hybrid Microparticles (DCHM).

DCHM were synthesized via the sol-gel method from a bridged precursor of the type $(H_3CO)_3$ – Bridge – $(OCH_3)_3$. The bridged precursor was obtained from an epoxy-amine reaction between an organically modified silane (3-glycidoxypropyltrimethoxysilane, GPMS) and dodecylamine (DA), by employing a molar ratio GPMS/DA = 2, under N_2 atmosphere at 70º C for 48 h. The hydrolytic condensation of the precursor was accomplished at room temperature in a mixture of THF/n-Hexane (1/2 volume ratio) as reaction solvent. Formic acid (FA) was used as catalyst under the following molar ratios: FA/Si = 3 and H_2O/Si = 1.05. Aspirin (AS) was used as model anti-inflammatory drug, being dissolved in the THF/n-Hexane mixture together with the obtained bridged precursor (employing a GPMS/AS = 2 molar ratio). As the catalyst was added dropwise, ultrasonic irradiation was continuously applied to the system. A 6-mm diameter ultrasonic tip Sonic Vibra-Cell (130W/20kHz) was employed as irradiation source with 50 % power intensity, up to a phase separation appeared as a white suspension. The solid was filtered and the remaining solvent evaporated at 80º C. The resulting powder was heated in an oven at 110º C for 3 h. The final powder was characterized by FTIR spectroscopy, SEM microscopy, ^{29}Si NMR and small angle X-ray scattering (SAXS).

In Vitro Cytotoxicity Tests of the DCHM.

Cell response was assessed *in vitro* according to the ISO 10993-5 specification (International Standard ISO 10993-5 – Biological evaluation of medical devices – Part 5: Test for *in vitro* cytotoxicity). Cells (CHO-K1 cell line, micoplasm-free certificated) were incubated with extracts of the DCHM in order to determine the potential toxicological hazard. After 72 h of incubation time at 37º C under 5 % CO_2, the biological response was evaluated. The morphology (general aspect, vacuolization, adhesion, lysis, membrane integrity) was evaluated in comparison with a control sample (which was not exposed to the extract).

Preparation of Calcium Phosphate Cements (CPCs) containing DCHM.

Four CPCs formulations were prepared from an equimolar mixture of synthesized tetracalcium phosphate (TTCP) and commercial dicalcium phosphate anhydrous (DCPA, Aldrich). Details of the tetracalcium phosphate synthesis are described elsewhere [5]. The cements contained different amounts of DCHM: (1) without incorporating DCHM (control sample), (2) incorporating 1 wt % of DCHM, (3) incorporating 5 wt % of DCHM and (4) incorporating 10 wt % of DCHM. In each case, a 5 vol % oxalic acid solution was used as liquid phase of the formulations.

The cement systems were prepared by mixing 1.3 g of solid phase and the required amount of liquid phase to form a workable paste. After that, the pastes were incubated at 37º C and 100 % relative humidity. In each case, the initial and final setting times were measured by employing the Gillmore needles technique (ASTM, C266-99). After 24 h of incubation time, the hardened samples were removed from the moulds to be characterized by XRD, FTIR spectroscopy and SEM microscopy.

Results and Discussion

Synthesis of DCHM.

Fig. 1 shows a SEM image (a) and the FTIR spectrum (b) corresponding to the synthesized DCHM. Monodispersed microspheres were obtained via ultrasonic irradiation-assisted hydrolytic condensation of the synthesized bridged precursor. A mean particle size of 2.4 ± 0.1 μm was determine from image analysis.

The FTIR spectrum confirms the effective incorporation of AS inside the microspheres. The bands located at 1740 cm^{-1}, 1290 cm^{-1} and 1200 cm^{-1} were assigned to functional groups corresponding to the AS molecule. The bands observed at 1040 cm^{-1} and 1120 cm^{-1} were attributed to Si-O-Si bonds in the inorganic structure of the hybrid material. The presence of the band located at about 905 cm^{-1}, corresponding to Si-OH groups, is indicative that the polycondensation was incomplete. The broad band at 3400 cm^{-1} corresponds to the OH groups present in the organic bridging chains.

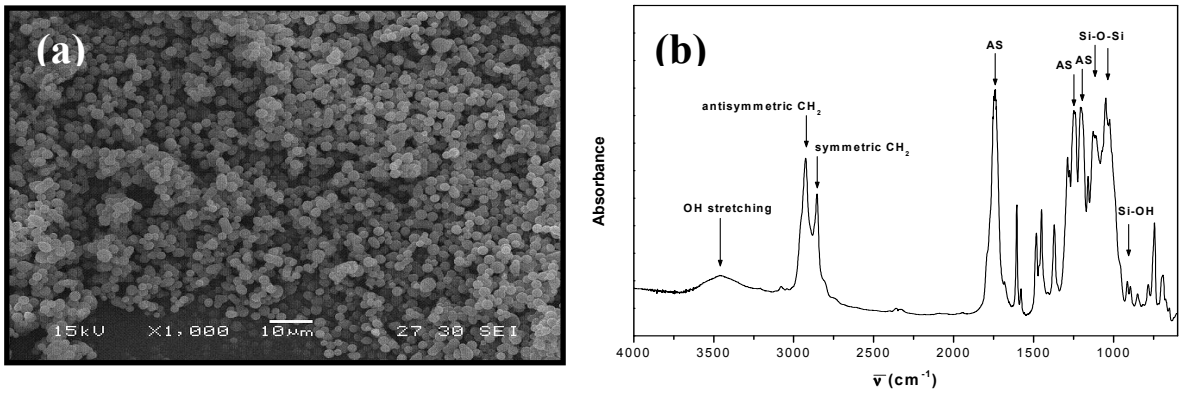

Fig. 1 SEM image (a) and FTIR spectrum (b) corresponding to the aspirin-containing hybrid microspheres.

The conversion into Si-O-Si bonds reached for the inorganic polycondensation was 89.5 %, as determined from ^{29}Si NMR spectrum. This is in agreement with the incomplete condensation inferred from FTIR results. The SAXS analysis of the obtained microspheres revealed the presence of a diffraction pattern located at q_{max} = 0.20 Å^{-1} which represents a correlation length of 31.4 Å. Experimental evidence obtained from SAXS, ^{29}Si NMR, FTIR techniques suggested that the basic structure of ordered domains consisted of hybrid organic-inorganic multilayers separated by hydrophobic regions with a thickness equal to the length of a tail-to-tail association of dodecylamine chains in all-trans conformations.

***In Vitro* Cytotoxicity Tests of the DCHM.**

Fig. 2 depicts two microphotographs corresponding to the cells cultured in the extracts after 72 h at 37º C and 5 % CO_2: (a) Control and (b) DCHM-extract.

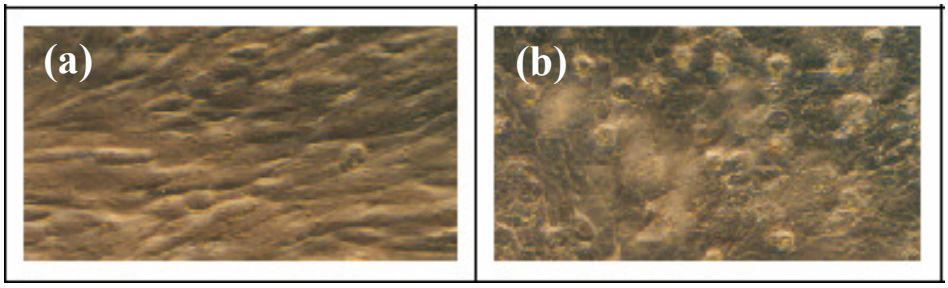

Fig. 2 Cultured cells: (a) Control, (b) DCHM-extract.

In the control sample, cells CHO-K1 showed the formation of a monolayer with normal both growth and phenotype. On the other hand, a monolayer with some changes in composition was observed when cells were exposed to the DCHM-extract. Few cells became spherical; however, the attached cells maintained a similar phenotype as that corresponding to the control cells. As a result, this could be interpreted as practically non-cytotoxic behavior.

Preparation of Calcium Phosphate Cements (CPCs) containing DCHM.

The measured initial and final setting times were practically the same for all the prepared formulations. The determined range for the initial and final times results adequate for clinical applications in dentistry and bone restorations (12 min-20 min for the initial and final times, respectively). High degree of conversion to low-crystallinity HA was evidenced from XRD analysis for all the prepared cements.

Fig. 3 shows the SEM micrographs corresponding to the developed bone cements with different content of DCHM, in comparison with the control cement.

The microstructural analysis revealed a progressive increase in the final porosity of the cements as the content of DCHM increased. The presence of microparticles led to an interconnected porosity, mainly in the 10 wt % sample. It is known that, the higher the porosity of the cement bodies the

lower the resulting mechanical strength, however, a high degree of pore interconnectivity is beneficial to get an adequate infiltration of blood vessels and bone cells. On the other hand, it is well known that interconnected pores (~ 100 μm) promote both resorption processes and growth of new bone tissue.

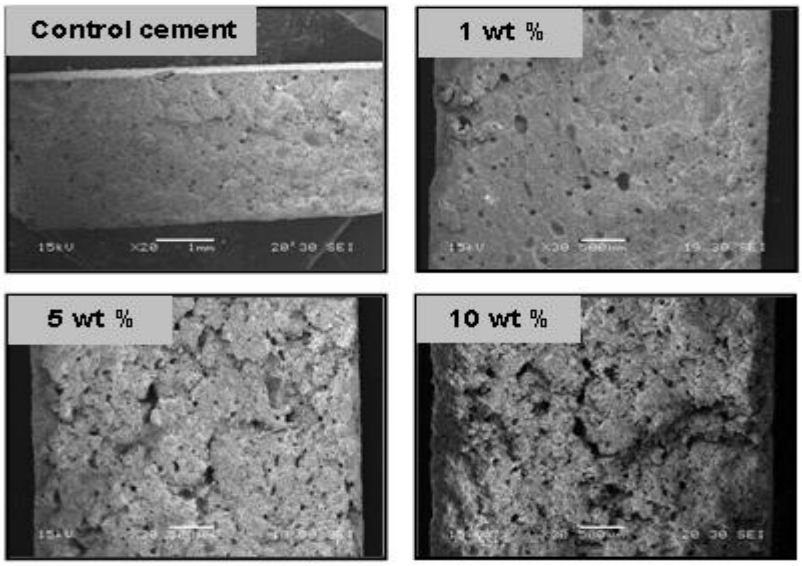

Fig. 3 SEM micrographs corresponding to the developed cements.

In the cements developed in this work (mainly the 5 and 10 wt % content-DCHM samples), interconnected macropores of about 200 μm were generated. These are promising results in order to employ these calcium phosphate cements in bone restorations under low mechanical stresses.

Conclusions

Novel CPCs incorporating DCHM were developed from hydraulic pastes constituted of synthesized TTCP, commercial DCPA and AS-containing hybrid microparticles. Monodispersed nanostructured hybrid microspheres containing AS were synthesized via ultrasonic irradiation-assisted sol-gel method. These microspheres act as drug delivery as the setting process of the cements proceeds. Cytotoxicity tests revealed that the DCHM have a practically non-cytotoxic behavior, which is a promising result for the microparticles to be used in tissue-contact sites.

The range of setting time values (12 min-20 min for initial and final times, respectively) results adequate for clinical applications in dentistry and bone restorations. The presence of DCHM did not modify neither the degree of conversion to low-crystallinity HA nor the measured setting times of the CPCs, however, the amount of incorporated microparticles considerably affected the degree of porosity (macropores of 200 μm) and interconnectivity of the cement matrix. This could be very useful in order to obtain both an adequate resorption process and growth of new bone tissue.

Drug delivery tests from the synthesized AS-containing microparticles are currently in progress.

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