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High-frequency impedance measurement as a relevant tool for monitoring the apatitic cement setting reaction

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ABSTRACT

This work reports the development of a relevant and general method, based on high-frequency impedance measurements, for the *in situ* monitoring of the alpha-tricalcium phosphate to calcium-deficient hydroxyapatite transformation that is the driving force of the hardening processes of some calcium phosphate cements (CPCs) used as bone substitutes. The three main steps of the setting reaction are identified in a non-invasive way through variation of the dielectric permittivity and dielectric losses. The method is also likely to characterize the effect of the incorporation of additives (i.e. antiosteoporotic bisphosphonate drugs such as Alendronate) in the CPC formulation on the hydration process. It allows us not only to confirm the retarding effect of bisphosphonate by an accurate determination of the setting times, but also to assess the phenomena that take place when Alendronate is added in the liquid phase or combined to the solid phase of the cement composition. Compared to the conventional Gillmore needle test, the present method offers the advantage of accurate, user-independent, in situ and real-time determination of the initial and final times of the chemical hardening process, which are important parameters when considering surgical applications.

1. Introduction

The discovery of calcium phosphate cements (CPCs) constitutes a significant advance in the field of biomaterials for bone reconstruction, especially for injectable compositions which allow implantation using minimally invasive surgical techniques [1–5]. These formulations most often consist of combinations of calcium orthophosphates. They have been extensively developed to obtain biocompatible materials showing improved mechanical properties in comparison to ceramics, while preserving their ability to be resorbed in vivo and replaced over time by newly formed bone. To be of practical use in a surgery room, the cements must reach a suitable mechanical strength after an acceptable time. Indeed, from the moment it is prepared, the cement paste changes from a fluid to a rigid state until its plasticity is completely lost and a specific mechanical resistance is reached (generally between 5 and 50 MPa in compression). Throughout this setting period, there is a time window during which the cement can be handled (e.g. for surgical implantation). The hardening process then continues while the mechanical strength of the cement paste gradually increases. Therefore, determining the initial and final setting times of injectable bone cements is of great importance. For this purpose, two standardized methods are classically used: the Gillmore needles [6,7] or Vicat needle [8] tests. These methods are based on the concept of "visible indentation", which is operator-dependent and can result in poor reproducibility from one research group to another. Furthermore, the methods are unable to provide information about the progress of the chemical reaction controlling the setting process. Monitoring chemical characteristics (i.e. nature of the products and their relative amount) and physical properties (i.e. porosity and mechanical strength) of the cement as a function of time most often entails the collection of samples at regular intervals and requires rather sophisticated characterization techniques, including X-ray diffraction, solid-state NMR, FTIR, mercury intrusion porosimetry, calorimetric analyses, scanning electron microscopy, compressive strength measurements or gas adsorption/desorption for surface area determination. In all cases the conditions are destructive, since the samples are, for example,

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soaked in acetone to quench the setting reaction, especially in its early stage.

The design of analytical methods allowing non-destructive and in situ dynamic monitoring of the setting reaction of cements dedicated to orthopedic applications is thus of great interest. In this context, ultrasonic techniques have been proposed to monitor the change in the viscoelastic properties of hydroxyapatite–polymethylmethacrylate systems [9] and calcium sulphate-based bone cements [10], but the high sound attenuation induced by the high content of liquid in the initial cement paste largely limits the potential of such techniques to investigate the initial stages of the setting reaction. On the other hand, impedance spectroscopy based on the electrical properties of the studied medium was exploited as a non-destructive technique for studying hydration processes occurring in other kinds of cement-based materials, even for low conductivity media like mortars [11–18]. Several approaches have been developed to correlate the electrical parameters with the mechanisms related to dissolution–precipitation reactions and microstructure changes occurring in Portland cement. They include DC electrical conductivity [11,12], measurements of the reflection coefficient of an electromagnetic wave sent perpendicularly to the flat surface of the sample [13–15] and impedance spectroscopy in the low frequency range (with major contributions of ionic species and charges present on the surface of particles) [16] or in the high frequency range (where the response is essentially related to the behaviour of the solid/liquid interface and structural characteristics) [17,18].

Computer models have been proposed to simulate the electrical response of cement pastes with equivalent circuits and to discriminate the contribution of the solid phase from that related to the electrolyte filling the material pores [19–21], or to distinguish between free water and water molecules either hydrogen-bonded to the surface of the cement particles or combined to hydration products [14,15,22–25]. Although the literature contains a number of contributions of the dielectric technique for the characterization of concrete, mortar and similar materials, only a few articles report on monitoring the hydration process of calcium phosphate cements by AC impedance, and these have been restricted to the low frequency range (kHz). For instance, Liu and co-workers [26,27] demonstrated that the introduction of crystal seeds accelerates the setting reaction but concomitantly reduces the compressive strength of CPCs. Other studies have focused on the relationship between the dielectric response of hydroxyapatite (HA) and its structure by considering the influence of temperature, the porosity and degree of hydration of HA, or the frequency of the applied electrical field [28–30].

In this paper, we propose to demonstrate that high-frequency impedance measurements provide an efficient way to monitor in real time the alpha-tricalcium phosphate (α-TCP) to calcium-deficient hydroxyapatite (CDA) transformation, which is the driving force of the setting reaction of some apatitic cements. Indeed, this technique in such a frequency range was previously reported to be able to show chemical changes at solid/liquid interfaces [31–35]. Recently, preliminary observations have demonstrated that significant variations of the dielectric parameters took place during the hardening process of a bisphosphonate (BP)-modified CPC when the frequency range of the applied electrical field was between 0.4 and 100 MHz [36]. This original approach enables us to investigate under non-destructive conditions, very close to the situation of *in vivo* implantation (body temperature, humid environment), the influence of additives (i.e. Alendronate) depending on the way they are introduced into the formulation. The evolution of dielectric parameters for data collection in this high frequency range allows us to assess the change in the chemical composition of the cement during the reaction, as well as the initial and final setting times.

2. Experimental

2.1. Calcium phosphate cement

The apatitic CPC is a mixture of 78 wt.% α-TCP ($Ca_3(PO_4)_2$), 5 wt.% dicalcium phosphate dihydrate (DCPD, $CaHPO_4{\cdot}2H_2O$; Fluka), 5 wt.% monocalcium monohydrate (MCPM, $Ca(H_2PO_4)_2{\cdot}H_2O$; Fluka), 10 wt.% CDA ($Ca_{10-x}[\ ]_x(HPO_4)_y(PO_4)_{6-y}(OH)_{2-z}[\ ]_z$), prepared as described previously [37], and 2 wt.% hydroxypropyl methyl cellulose (E4M®, Colorcon-Dow Chemical, Bougival, France).

Alendronate-doped CDA was obtained by suspending 1 g of CDA in 10 ml of an aqueous Alendronate solution ([Alendronate] = 1.23×10^{-2} M– Alendronate sodium trihydrate (Sigma–Aldrich)), as previously reported [36]. The resulting doped CDA was then collected by centrifugation, rinsed with water and dried at room temperature to a constant weight before use.

Each CPC powder was milled for 30 min to obtain a similar particle size distribution, controlled using a Beckman-Coulter LS 230 laser granulometer. This grinding step leads to an almost complete transformation of DCPD into $CaHPO_4$ (DCPA) and of MCPM into $Ca(H_2PO_4)_2$ (MCPA), as observed by ^{31}P MAS(magic angle spinning) and CP (cross-polarization) MAS NMR measurements.

All cement paste samples were prepared by mixing 8 g of the powdered preparation with 4 ml of a 5 wt.% Na_2HPO_4 (Fluka) aqueous solution in a specific syringe (Fig. 1C) for 2 min to ensure the homogeneity of the obtained paste before analysis. This liquid/solid ratio ($R = 0.5$ ml g^{-1}) was found to be optimal for providing suitable injectability and resorbability for its practical use in orthopedic surgery.

2.2. Instrumentation

The high-frequency impedance measurements were recorded between 0.4 and 100 MHz, using an HP 4194 A impedance/gain-phase analyser (Hewlett–Packard). Our experimental set-up allowed us to concomitantly perform complex impedance and Gillmore needles measurements at 37 °C. The thermostated axial capacitive cell and the thermostated cell for Gillmore measurements were home-made (Fig. 1). The bottom of the dielectric cell was first filled by injecting, via a syringe, 4–5 ml of the cement preparation, which was then covered with 5 ml of a 0.9 wt.% NaCl aqueous solution to mimic a real-life implantation. It was verified that the NaCl solution added did not affect the impedance response and that the quantity of the cement paste introduced into the dielectric cell was sufficient to avoid disturbing the dielectric measurements by contributions other than those of the cement paste. The top of the cell was closed to avoid evaporation. The remaining paste was deposited in the second cell for the Gillmore test, so that comparison of the dielectric and Gillmore results could be strictly made on the same sample and under the same experimental conditions. Each experiment was monitored until stabilization of the values of each dielectric parameter was observed (i.e. experiments were conducted for up to 12 and 36 h for undoped and Alendronate-modified cement, respectively). The experiments were run twice and led to similar results, giving evidence of the good reproducibility and accuracy of this technique.

The experimental device was completed by a computer allowing automatic data acquisition and real-time calculation of the complex impedance, Z^*, from which the dielectric permittivity, ε' (related to dipole variation), and dielectric losses, ε'' (related to the motion of free charges), were computed [38]. The evolution of $-Z''$ vs. Z' (the imaginary part and the real counterpart of Z^*, respectively) is called the Nyquist plot.

For the Gillmore standard method, the initial setting time (t_i) is defined as the time elapsed until the small needle (diameter