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Development of LiCl-containing calcium aluminate cement for bone repair and remodeling applications

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ABSTRACT

The effect of LiCl additions on the in vitro bioactivity, hemolysis, cytotoxicity, compressive strength and setting time of calcium aluminate cements was studied. Calcium aluminate clinker (AC) was obtained via solid state reaction from reagent grade chemicals of $CaCO_3$ and Al_2O_3. Calcium aluminate cements (CAC) were prepared by mixing the clinker with water or aqueous LiCl solutions (0.01, 0.0125 or 0.015 M (M)) using a w/c ratio of 0.4. After 21 days of immersion in a simulated body fluid (SBF) at physiological conditions of temperature and pH, a Ca-P rich layer, identified as hydroxyapatite (HA), was formed on the cement without LiCl and on the cement prepared with 0.01 M of LiCl solution. This indicates the high bioactivity of these cements. The cements setting times were significantly reduced using LiCl. The measured hemolysis percentages, all of them lower than 5%, indicated that the cements were not hemolytic. The compressive strength of the cements was not negatively affected by the LiCl additions. The obtained cement when a solution of LiCl 0.010 M was added, presented high compressive strength, appropriated bioactivity, no cytotoxicity and low setting time, making this material a potentially bone cement.

1. Introduction

One of the most important properties of an implant is biocompatibility. A bioactive material is able to form a direct bond with the osseous tissue through the formation of an apatite layer on its surface when it is in contact with a physiological fluid [1–3]. Hydroxyapatite (HA) is a biocompatible and bioactive material [4]. However, some medical applications require materials with higher mechanical properties than HA and able to be molded in situ and bone cements possess these characteristics [5]. Calcium phosphate cements have been deeply studied. Most of these cements show a compressive strength within a range of 10 to 40 MPa using no reinforcement, such as organic materials or fibers [6].

Bone cements currently used for implant fixation are based on polymethyl-methacrylate (PMMA). However, these cements have some limitations, mainly: *i)* generation of high temperatures during their hardening which can causes cell death, *ii)* gas releasing and *iii)* no chemical bonding to bone leading, in some cases, to cement fractures [7,8].

A possible alternative to PMMA cements are calcium aluminate cements (CAC). These have some attractive characteristics, such as high mechanical properties and low setting temperatures [9]. Compared with the calcium phosphate cements, the main advantage of CAC are their mechanical properties, which are considerable higher. However, this kind of cements presents long setting times, which limits their use in some medical applications [10]. S. H. Oh et al. [11] studied the effect of the addition of LiF in a CA-PMMA composite cement observing that bioactivity was improved. These authors also studied the effect of the addition of LiF and maleic acid on the CAC properties finding that the cement with 0.5 g of LiF and 8.75 g of maleic acid showed high compressive strength (after one day of curing in distilled water), short setting time and no cytotoxicity [12]. There are also some other additives that have been used to reduce setting time.

T. Matusinović et al. studied the effect of some lithium salts on the setting time of high alumina cements (HAC) finding that this time was significantly reduced when small additions of these salts were used [13].

B. R. Currell et al. evaluated the effect of different additives such as LiCl, NaCl, KCl and NaOH reporting that the setting time of HAC is influenced by these substances [14]. Lars Kraft mentioned in studies that additions of lithium salts (30–90 ppm) in the mixed solution accelerate the hardening process of CAC [15].

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Ukrainczyk et al. [16] studied the effect of different salt chlorides additions on the hydration of CAC. They observed that LiCl accelerated the setting time when very low additions were used. A strength development at early ages was also observed.

However, neither the compressive strength (after different periods of immersion in SBF) nor hemocompatibility of CAC with LiCl additions have been evaluated.

In this work the effect of LiCl additions on the setting time, compressive strength, bioactivity and hemolysis of CAC was studied.

2. Experimental

2.1. Clinker synthesis

The calcium aluminate clinker was obtained by solid state reaction from a mixture of reagent grade chemicals of calcium carbonate ($CaCO_3$) and alumina (Al_2O_3). The mixture was homogenized in a plastic jar with alumina balls in acetone for 4 h using milling rods, then the mixture was dried at 80 °C, de-agglomerated and heat treated at 1450 °C for 4 h. The obtained clinker was milled in a porcelain jar with alumina balls for 2 h, reaching an average particle size of 22 μm. The clinker was analyzed by X-ray diffraction (XRD; Philips Xpert 3040e).

2.2. Cement preparation

The LiCl aqueous solutions (0.01, 0.0125 and 0.15 M) used were obtained by dissolving appropriate amounts of LiCl in deionized water. Different cements were prepared by mixing the calcium aluminate clinker with the corresponding LiCl solution at a w/c ratio of 0.4 until a paste was formed. For comparative purposes, a paste was prepared using pure water. Sample identification and LiCl aqueous solution concentrations are shown in Table 1.

Pastes were casted into two different nylamid molds, the first of 10 mm in diameter and 2 mm in height for assessing in vitro bioactivity and cytotoxicity, and the second of 6 mm in diameter and 12 mm in height for compressive strength testing and hemolysis assays. All samples were set for 1 h.

2.3. Setting times measurement

Initial and final setting times of CAC were determined using a Vicat needle (ASTM C 191) [17]. The apparatus consisted of a movable rod (300 g, 10 mm diameter) and a steel needle (1 mm diameter, 50 mm length). CAC pastes, prepared as described in Section 2.2, were poured into conic molds and placed on base plates. Initial and final setting times were determined and temperature was recorded every 1 min.

2.4. In vitro bioactivity assessment

The simulated body fluid (SBF) used in these tests was prepared following the procedure described by Kokubo et al. [18]. After setting, samples were immersed in 200 ml of SBF in a polyethylene flask and placed into an incubator at 37 °C for 7, 14 or 21 days. After immersion, samples were stored in a desiccator. The surface of the cement samples was analyzed before and after immersion in SBF by scanning electron microscopy (SEM; Philips, XL30 ESEM), energy dispersive spectroscopy (EDS; EDAX, Pegasus) and XRD (XRD; Philips Xpert 3040e).

The Ca, P, Li and Al concentrations of the remaining SBFs were determined by inductively-coupled plasma atomic emission spectrometry (ICP-AES; Thermo Elemental, IRIS Intrepid II XSP); pH of the remaining SBFs was also monitored as a function of immersion time (Thermo Orion 420 pH meter).

Table 1
Aqueous LiCl solution concentrations used for preparing the cements (w/c = 0.4).

Sample ID	LiCl solution concentration (M)
CAC	0
CAC C1	0.010
CAC C2	0.0125
CAC C3	0.015

2.5. Compressive strength testing

Before compressive strength evaluation, samples were immersed in SBF in a polyethylene flask and placed into an incubator at 37 °C for 1, 7, 14 or 21 days. After immersion, samples were soaked in ethanol to stop the hydration reactions and stored in a desiccator. The compressive load was applied at a crosshead speed of 0.5 mm/min. The mechanical strength evaluation was performed according to the ASTM F 451 [19]. Five specimens were tested for each composition and the strength average and standard deviation were calculated.

2.6. Hemolysis testing

Fresh human blood from a healthy volunteer donor was obtained. The blood was collected in tubes with anticoagulant (EDTA) and centrifuged at 3200 rpm for 4 min. The obtained pellets were washed three times with phosphate-buffered saline (PBS). The supernatant was then removed and 1 ml of the purified erythrocytes was diluted 1:9 with PBS. The samples of cement to assess, prepared as described in Section 2.2, were hydrated in SBF for 24 h at 37 °C before testing. The hemolysis tests were performed placing the specimens into tubes with a volume of 1800 μl of PBS for 30 min at 37 °C in a water bath. Then, 200 μl of the blood solution were added to the tubes containing the specimens and these were incubated for 1 h at 37 °C in a water bath. Both positive and negative controls were prepared. The negative control (0% hemolysis) was prepared by adding an isotonic solution; in this case PBS was used. The positive control (100% hemolysis) was prepared by adding deionized water. Six experimental replicates were used for each group. After incubation, specimens were centrifuged at 3200 rpm for 4 min at room temperature and the supernatant was collected. The absorbance (A) value of the hemoglobin released from the erythrocytes was measured spectrophotometrically at 545 nm. Hemolysis percentage was calculated as follows [20]:

$$\text{Hemolysis}\ (\%) = \left[\left(A_{\text{sample}} - A_{\text{negative control}}\right)/\left(A_{\text{positive control}} - A_{\text{negative control}}\right)\right] \times 100 \quad (1)$$

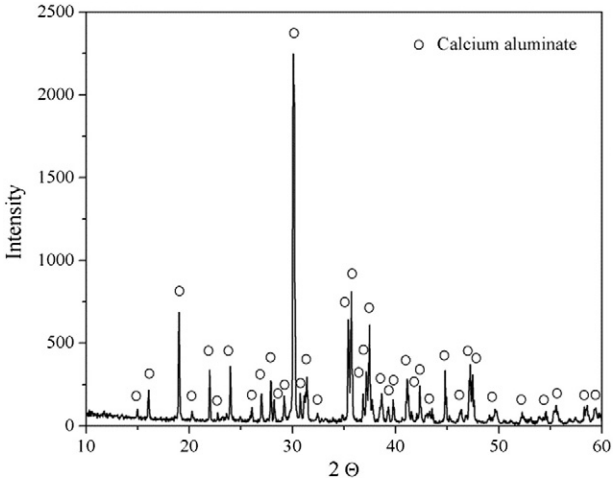

Fig. 1. XRD pattern of the CA clinker.