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CONTENT

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SURFACE MORPHOLOGY, COMPRESSIVE STRENGTH AND BIOCOMPATIBILITY OF CALCIUM ALUMINATE DENTAL CEMENT

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ABSTRACT

The aim of this study was to investigate the properties of experimental calcium aluminate (CA) dental cement, synthesized from $CaO \times Al_2O_3$ and $CaCO_3$. Calcium silicate (Portland cement, PC) served as a control. The elastic modulus and maximum stress obtained using the universal testing machine showed that CA has greater mechanical resistance than the control PC. Scanning electron microscopy (SEM) analysis of cements specimens before and after soaking in phosphate buffer saline showed that hydrated cements exposed crystal particles of calcite and new aluminum containing phases on their surfaces, suggesting their bioactive potential. Biocompatibility of the CA dental cement was evaluated by observing L929 cells morphology using phase-contrast microscopy. Cells treated with CA extract preserved their structural integrity without any changes in cell morphology, but with a slightly inhibited proliferation rate after 24h treatment, while PC induced changes typical for cell death.

INTRODUCTION

Dental materials engineering has gone through several phases of innovations following new demands that had been placed in front of the research community. In this sense, the concept of manufacturing biologically inert materials has been nowadays replaced with the idea to fabricate bioactive materials. In other words, instead of manufacturing the material that is inert in contact with the surrounding human tissues (i.e. titanium), current trends go in line with formulating the materials capable to provoke a positive response from the host tissue [1]. In the field of dentistry, the revolution occurred when a mineral trioxide aggregate (MTA) was introduced in the nineties at Loma Linda University. MTA is calcium silicate-based dental cement that was intended as a substitute for ~50 years gold standard – calcium hydroxide. Namely, although both materials are capable to release calcium ions and consequently neutralize acidic cariogenic environment, the effect of calcium silicates is measured in the terms of months and years in comparison to ~10 days of calcium hydroxide puferizing capacity [2]. Many calcium silicate-based formulations have been introduced and commercialized such as MTA Angelus (Angelus science and technology, Londrina, Brazil), ProRoot MTA (Dentsply, Tulsa, Oklahoma, USA), MTA Flow (Ultradent, Utah, USA), Bioaggregate (Innovative BioCeramix, Burnaby, Canada), Theracal LCR (Bisco Dental, Illionois, USA), iRoot (IBCeramix, Vancouver, Canada), Endo CPM (Egeo, Buenos Aires, Argentina), Biodentine (Septodent, Saint Maur, France) etc. [1].

Another advantage of calcium silicate cement over calcium hydroxide one is its superior physical properties, i.e. compressive stress of >20 MPa [3,4]. Therefore, recent efforts are directed toward using calcium aluminate-based cements (CA) since they have superior physical properties [5,6]. However, it is not known if the aluminum incorporation can influence cement's biological behavior as well as if the interaction with biological fluids can result in the formation of the apatite-like crystals on its surface. Thus, the aim of this study was to investigate the morphological features on

the surface of hydrated CA cements, cements elastic modulus and maximum stress and their effect on the morphology of L929 cells.

METHODS

Synthesis of experimental calcium aluminate cement - Experimental cement was synthesized from CAC ($CaO \times Al_2O_3$) and calcite ($CaCO_3$) using the protocol described in detail elsewhere [5]. In brief, for the synthesis of CAC, pseudo boehmite sol (AlOOH) was used. Aluminum tri-sec-butoxide was dissolved in a mixture ethanol/water (1:4), wherein a molar ratio of aluminum trisec-butoxide in a mixture ethanol-water was 1:50. This mixture was then heated at 85°C under vigorous stirring. After 2 h of heating, the solution was cooled to room temperature and the sulfuric acid was added dropwise to reach the molar ratio H_2SO_4/aluminum tri-sec-butoxide of 0.04. Calcium chloride tetrahydrate ($CaCl_2x4H_2O$) (Sigma-Aldrich, St. Louis, MO) was used as the precursor for the production of $CaCO_3$. Briefly, the amount of 5 mmol of $CaCl_2x4H_2O$ was dissolved in 50 ml of ethylene glycol (Sigma-Aldrich) and sonicated at 40°C (Elmasonic S_3OH). Afterward, 10 mmol of sodium bicarbonate ($NaHCO_3$) (Sigma-Aldrich), dispersed in 50 ml of ethylene glycol, was added dropwise for 30 min, upon mechanical stirring. Pure calcium silicate – Portland cement (PC, Italcementi, Bergamo, Italy) served as a control material.

Determination of elastic modulus and maximum stress - The elastic modulus and maximum stress were determined in accordance with International Standard Organization (ISO) 6876 by dividing the breaking load (unit: Newton) of every specimen measuring 8 mm in diameter and 1 cm in height on a universal testing machine (Instron 1332, Norwood, USA, loading rate 50 N/min) by the cross-sectional area (Newton per square millimeter). Five specimens per group were tested.

Scanning electron microscopy (SEM) analysis of cements surface morphology - Specimens measuring 5 mm in diameter and 1 mm in height were immersed in phosphate buffer saline (PBS) and placed in an incubator at 37°C for 14 days. Afterward, the specimens were dried, gold coated, and visualized under scanning electron microscopy (JEOL JSM-7500).

Cell culture and treatment - For *in vitro* analysis cements were manipulated under sterile conditions. After mixing, CA and PC cements were placed into pre-sterilized PTFE moulds (5 mm in diameter and 3 mm thick) to set for 24 h in a humidified atmosphere. Specimens were sterilized by ultraviolet irradiation for 2 h and thereafter immersed in 1 ml complete medium Dulbecco's modified Eagle medium (DMEM; Gibco, Thermo Fisher Scientific, Inc., Waltham, MA, USA) supplemented with 5 % fetal bovine serum (FBS), 2 mM L-glutamine and penicillin/streptomycin (all from Capricorn Scientific, Ebsdorfergrund, Germany). After 24 h incubation at 37^{o}C discs were discarded and the supernatants (extracts) were collected for cell treatment.

The mouse fibroblast L929 cell line (European Collection of Animal Cell Cultures, Salisbury, UK) was cultivated in a complete medium and maintained at 37°C, in a humidified atmosphere with 5 % CO_2. Cells were prepared for experiments using the conventional trypsinization procedure with trypsin/EDTA and seeded in 96-well flat-bottom plates (5×10^3 cells/well) for the cell morphology assessment. Cells were treated 24 h post-seeding with pure cements' extracts. Morphological changes were evaluated after 24 h of treatment by phase-contrast light microscope Leica DCF320 (Leica Microsystems DMIL, Wetzlar, Germany). Phase-contrast light microscopy images were taken at 3 random positions within each well at 200× magnification with Leica Microsystems DFC320 camera and Leica Application Suite software (version 2.8.1).

RESULTS AND DISCUSSION

The maximum stress calculated in strain/stress diagram of the experimental CA cement (36±4 MPa) was superior in comparison to that in the PC (12±3 MPa). Elastic modulus of CA cement was 2400±100 MPa, while elastic modulus of PC was 1300±100 MPa. The force to displacement curves

showing maximum loading force are presented in Figure 1. As expected, CA cement showed 3 times greater mechanical resistance than PC and therefore it may be used in clinical applications when the material is subjected to high mechanical stress such as in indirect restorations or when it is used as a direct pulp capping material. In addition, MTA is often used to heal huge bifurcation lesions [2,6]. In this case, the material also suffers great vertical stress which may lead to its fracture and spreading of the infection. It may be assumed that mechanically superior CA cement may be more suitable in this clinical situation.

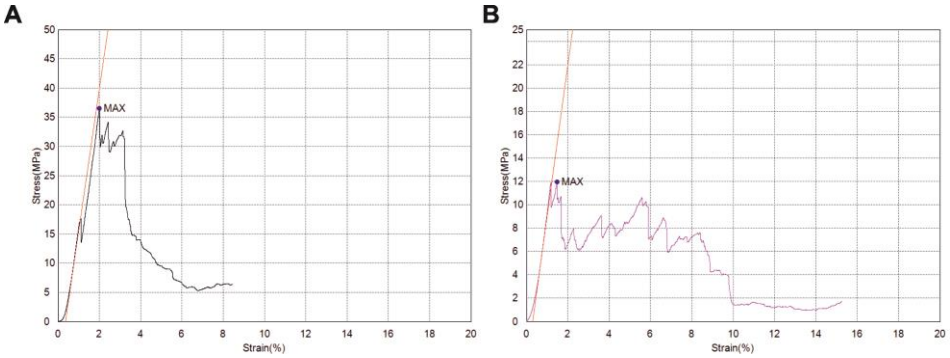

Figure 1. Representative diagram illustrating the amount of force required to fracture A) calcium aluminate and B) Portland cement specimens.

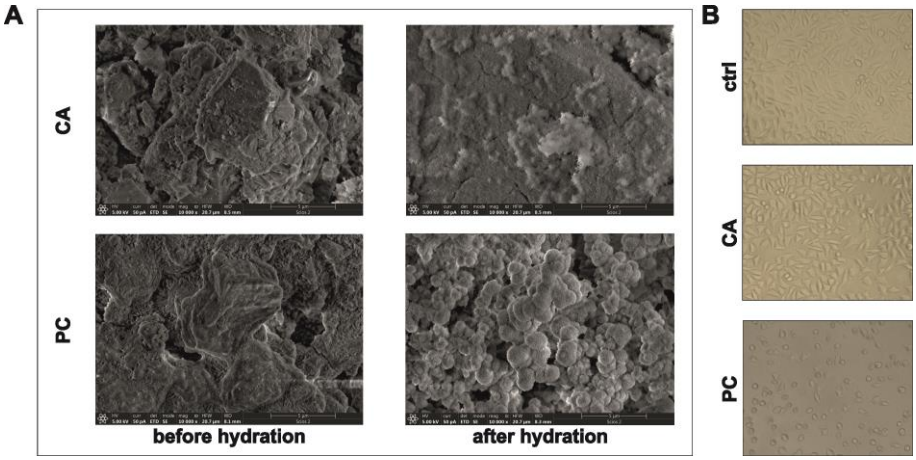

Figure 2. SEM images of cement surfaces and light microscopy images of L929 cells treated with cement extracts. (A) Morphological features of surfaces of calcium aluminate (CA) cement and Portland cement (PC) specimens before and after hydration in PBS. Scanning electron microscopy, magnification 10000×. (B) Effects of CA and PC pure extracts on the morphology of mouse fibrosarcoma cell line L929 in comparison to untreated, control cells (ctrl). Phase-contrast light microscopy (magnification 200×). The data are presented as pictures from one representative of three independent experiments.

The SEM analysis of CA cement showed that, before hydration, the polygonal crystals of calcite measuring 20 μm – 100 μm are observed. In hydrated cement, it can be noticed that morphology has turned mostly into needle-like crystals presumably indicating the formation of new aluminum containing phases (C_3AH_6 and aluminum hydroxide). In PC, the bioactivity of the material is clearly confirmed by changing the shape of the crystals from polygonal (unhydrated) one to globular one after hydration (**Figure 2A**). Similar behavior to PC is reported in the literature for Biodentine [7].

Light microscopy images of L929 cells treated with CA pure extract showed preserved structural integrity and cell morphology after 24h, but with slightly reduced proliferation. Contrary, PC triggered morphological changes typical for cell death – cell shrinkage and rounding and detachment of cells from the bottom well (**Figure 2B**). It may be the consequence of "non-purity" of the PC, because the employed PC was not calcium silicate cement synthesized in the laboratory, but commercially available material of the natural origin that may include in traces elements such as cobalt, mangane and iron [8].

CONCLUSION

Bearing in mind the results presented, CA dental cements have significant potential to become a suitable alternative to calcium silicate cements in dentistry. They showed the surface morphology capable to trigger cell spreading and proliferation. The pure extract of CA cement seems to be non-cytotoxic in L929 cell culture, conversely to PC which induces morphological changes typical for cell death. Superior elastic modulus and maximum stress of CA cements may present a huge advantage in some clinical situations. Further studies are needed to investigate in detail the full potential of CA cements in dentistry.

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