



In-vitro bioactivity, biocompatibility and dissolution studies of diopside prepared from biowaste by using sol–gel combustion method



Rajan Choudhary^a, Jana Vecstaudza^b, G. Krishnamurthy^c, Hanumantha Rao Balaji Raghavendran^c, Malliga Raman Murali^c, Tunku Kamarul^c, Sasikumar Swamiappan^{a,*}, Janis Locs^b

^a Department of Chemistry, School of Advanced Sciences, VIT University, Vellore - 632014, Tamil Nadu, India

^b Rudolfs Cimdins Riga Biomaterials Innovations and Development Centre, Institute of General Chemical Engineering, Faculty of Materials Science and Applied Chemistry, Riga Technical University, Riga, Latvia

^c Tissue Engineering Group (TEG), Department of Orthopaedic Surgery (NOCERAL), Faculty of Medicine, University of Malaya, Kuala Lumpur, Malaysia

ARTICLE INFO

Article history:

Received 13 January 2016

Received in revised form 21 April 2016

Accepted 27 April 2016

Available online 4 May 2016

Keywords:

Diopside

Topic:

Eggshell

Fuels

Apatite

Topic:

SBF circulation

Bone marrow cells.

ABSTRACT

Diopside was synthesized from biowaste (Eggshell) by sol–gel combustion method at low calcination temperature and the influence of two different fuels (urea, L-alanine) on the phase formation temperature, physical and biological properties of the resultant diopside was studied. The synthesized materials were characterized by heating microscopy, FTIR, XRD, BET, SEM and EDAX techniques. BET analysis reveals particles were of submicron size with porosity in the nanometer range. Bone-like apatite deposition ability of diopside scaffolds was examined under static and circulation mode of SBF (Simulated Body Fluid). It was noticed that diopside has the capability to deposit HAP (hydroxyapatite) within the early stages of immersion. ICP-OES analysis indicates release of Ca, Mg, Si ions and removal of P ions from the SBF, but in different quantities from diopside scaffolds. Cytocompatibility studies on human bone marrow stromal cells (hBMSCs) revealed good cellular attachment on the surface of diopside scaffolds and formation of extracellular matrix (ECM). This study suggests that the usage of eggshell biowaste as calcium source provides an effective substitute for synthetic starting materials to fabricate bioproducts for biomedical applications.

© 2016 Elsevier B.V. All rights reserved.

1. Introduction

By assisting body's response to self-repair, biomaterials were also found to assist in regenerating the tissues when stem cells are transplanted [1]. Scientists are developing novel and improved biomaterials to replace diseased or damaged tissues, cells and even entire injured body parts for regenerative medicine. It is a great challenge to extend the functionalities of biomaterials. For the past few decades, new and cost effective technologies have been adopted for the production of calcium based biomaterials from biowaste (e.g. eggshells) [2]. It has been found that a dry eggshell contains approximately 94–96% calcium carbonate. Globally, tons of hen eggs are produced to satisfy the increasing demands of food and nutrition manufacturing industries [3]. After usage of eggs on a large scale, the shells are considered as waste. This eggshell waste is either left as such in the open environment to decay or employed for land fillings. This unintentional motive leads to the soil as well as environmental pollution. In order to avoid such man-made damages to the natural environment, high emphasis is given in the conversion of these wastes into useful products. Reuse of such biowastes has proved to be an efficient method to prevent

pollution and landfills [4]. The concept of recycling biowastes has been employed for the preparation of different calcium containing bioceramics such as wollastonite, akermanite, larnite, hydroxyapatite, tri-calcium phosphate and their biological behavior has been studied [5–9].

In early 1970's, L.L. Hench introduced a new class of surface reactive silicates that can form chemical interfacial bonding between neighboring host tissues and implant [10]. The deposition of hydroxycarbonate apatite layer (HCA) on the implanted surface of the bioactive silicate was observed which induces the bone healing and stimulates bone regeneration [11]. Compatibility of bioactive silicates with tissues, physiological body environment, and their excellent mechanical strength makes them potential candidates for several applications in the field of hard tissue engineering [12,13]. Literature survey reveals that wollastonite, larnite, bregidite, forsterite, akermanite, diopside, merwinite etc. have been studied for dental restoration, bone substitutes, drug delivery system and tissue engineering applications. Due to the advanced bio-functionalities such as improved mechanical properties, slow degradation, superior HAP deposition and excellent in vivo biocompatibility, diopside is preferred over other silicate and phosphate bioceramics [14, 15].

Studies indicate that the reason for low degradation of diopside in SBF is due to the presence of magnesium ion in the crystal lattice. The

* Corresponding author.

E-mail address: ssasikumar@vit.ac.in (S. Swamiappan).

bond energy of Mg–O is higher as compared to Ca–O bond, this makes crystal system more stable and prevents the rapid degradation of diopside [16]. Previous reports show that diopside can be used for load bearing applications as it has remarkable mechanical strength with a fracture toughness of $3.5 \text{ MPa m}^{1/2}$ and bending strength 300 MPa, which is double to that of the bone [17]. Whereas, the commercially available calcium phosphate bioceramic implants are found to possess poor mechanical properties and hence cannot be used for stress and load-bearing applications [18].

Different methods like co-precipitation process, sintering, sol–gel method, solid-solution are reported for the preparation of diopside. The diopside prepared through these methods is found to be a suitable material for hard tissue engineering applications [14,17,19–22]. Raw materials used in these methods were $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$, $\text{Mg}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$, dolomite powders ($\text{CaO} \cdot \text{MgO}$), TEOS (Tetraethyl orthosilicate), $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$, ethanol, CaCO_3 , MgO , SiO_2 . In 2016, Ghomi et al. synthesized diopside at 800°C by sol–gel method by using calcium nitrate as the calcium source. It was found that the low intensity diopside peaks got enhanced after sintering the scaffold at 1200°C [23]. However, these methods involve higher thermal treatment which affects the particle size, morphology, porosity and nature of the product [24].

In the current report, we have attempted to synthesize pure diopside by sol–gel combustion method by using two different fuels. Eggshell biowaste was used as a substitute for the calcium source. Sol–gel combustion method is cost effective, energy efficient, low temperature process and produces highly homogeneous product [25]. Evolution of large amount of gases (NH_3 , H_2O , and CO_2) during combustion process help to disperse heat, and avoids oxides from getting sintered [26]. These advantages make sol–gel combustion method as preferable one over conventional synthesis routes. The calcination temperature required for different fuels was optimized and influence of fuels on particle size, porosity, surface area and morphology was investigated. The single phase diopside obtained after calcination was subjected for cellular studies and HAP deposition ability in SBF under static and refreshing conditions.

2. Experimental procedure

2.1. Materials and methods

Raw eggshells, Magnesium nitrate LR (99.0% SDFCL), L-alanine AR (99.5% SDFCL), Urea, pure (99% HIMEDIA), Tetraethyl orthosilicate (TEOS) (98%, Acros Organics), Concentrated Nitric Acid LR (69–72%, SDFCL), Ethylene Diamine Tetra Acetic Acid LR (98.0%, SDFCL), Eriochrome Black-T AR (99%, SDFCL), Ammonia Solution Extrapure AR (25%, SRL), Ammonium Chloride GR (98.8%, MERCK), Sodium Chloride AR (99.9%, SDFCL), Sodium Bicarbonate AR (99%, Nice Chemicals), Potassium Chloride AR (99.5%, SDFCL), Di-potassium Hydrogen Orthophosphate AR (99.0%, SDFCL), Magnesium Chloride AR (99.0%, SDFCL), Concentrated Hydrochloric Acid LR (35–38%, SDFCL), Calcium Chloride AR (98%, Qualigen Fine Chemicals), Sodium Sulphate Anhydrous AR (99.5%, SDFCL) and Tris(hydroxymethyl)aminomethane AR (99.8%, SDFCL) were used in the present study.

2.2. Extraction of calcium from raw eggshell and estimation of calcium ions by EDTA titration

Eggshells were collected from VIT hostel mess and fresh uncrushed eggshells were separated from compressed, torn out or broken shells. These selected eggshells were washed manually under running distilled water to remove dirt particles deposited on the surface of eggshells. In order to eliminate microbial contamination and unnecessary protein coatings, the washed eggshells were boiled at $100\text{--}110^\circ\text{C}$ for about 2 h with continuous stirring using glass rod. After boiling, the eggshells were transferred to filter paper to remove water droplets from the surface and later subjected into hot air oven drying at 150°C until the shells

are completely dried. The dried raw eggshells were finally crushed and grinded to fine powders by using mortar and pestle. The eggshell powder was then characterized by XRD and FTIR spectroscopy to support the fact that calcium carbonate is the main constituent present in eggshells as reported earlier [6,7]. Eggshell solution approximately equivalent to 1 M calcium nitrate solution was prepared by the addition of 15 mL conc. nitric acid to 10 g of eggshell powder and the resultant solution was filtered and made up to 1 L by adding distilled water. The calcium ion concentration was estimated by EDTA titration by following the procedure described previously [5]. The concentration of calcium ion (Ca^{2+}) present in the eggshell solution was found to be 0.98 M.

2.3. Synthesis of diopside

Diopside was prepared by sol–gel combustion method using urea as a fuel is termed as EDU while for L-alanine as EDL. Eggshell solution, magnesium nitrate, TEOS, urea, L-alanine and conc. Nitric acid were used as starting materials. Stock solutions of magnesium nitrate (1 M), urea (2 M) and L-alanine (2 M) were prepared separately by using double distilled water in 100 mL standard volumetric flask. Equimolar concentration of eggshell solution and magnesium nitrate solution was mixed thoroughly in a beaker. L-alanine solution was pipetted out from stock solution and transferred into the beaker with uniform stirring to obtain homogeneous mixture of starting materials. A clear solution was obtained by adding 2 M TEOS to the beaker. Similar experimental procedure was carried out in another beaker except that urea was used as a fuel in place of L-alanine. The solutions were stirred vigorously until the disappearance of transparent layer. The pH of reaction mixture in both beakers was adjusted to 1 by dropwise addition of conc. nitric acid to carry out the rapid hydrolysis of TEOS [27]. Beaker containing urea as the fuel took 2 h to form viscous gel at room temperature by using magnetic stirrer whereas the beaker containing L-alanine as a fuel took 17 h of stirring and half an hour heating at 40°C on magnetic stirrer to form a transparent gel. Thus, the gel formation time varies with fuel even though the composition of reaction mixture is same. This may be due to the formation of different polymeric network by different fuels. The gel containing beakers were kept undisturbed for 2–3 days to strengthen the gel and then dried at 150°C in hot air oven for 8 h to obtain blocks of solid masses. The dried gel masses were decomposed separately at 400°C for 1 h in preheated muffle furnace. Decomposition occurs in presence of oxygen to attain complete combustion of fuel by the oxidants. The resultant precursor contains residual carbon and nitrate groups as impurities. In order to eliminate these impurities the precursor was crushed to fine powder using agate mortar and pestle and calcined at 800°C (EDU) and 900°C (EDL) for 6 h in alumina crucibles to achieve the pure phase of diopside.

2.4. Material characterization

Heating Microscope with automatic image analysis (Heating microscope EM-201, Hesse instruments) was used to observe and characterize the sintering process in situ from room temperature till 1400°C . Prior to heating Microscopy analysis diopside powder samples were shaped by pressing into mold to obtain samples with correct shape for analysis. Pure phase identification of synthesized diopside was studied by X-Ray Diffractometer (Bruker, D8 advance, Germany), using $\text{CuK}\alpha$, Ni filtered radiation. Functional groups of synthesized diopside were examined by FTIR (IR Affinity-1, Shimadzu FTIR spectrophotometer) using KBr method. For FTIR analysis calcined diopside samples were grinded into fine powder separately using agate mortar and pestle. The fine diopside powder was mixed with KBr powders and again grinded. Finally, the diopside and KBr mixture was manually pelletized using pellet press. Scanning electron microscopy (SEM-CARL ZEISS) was used for morphological characterization and Energy dispersive X-ray spectroscopy (EDX- OXFORD Inc.) for elemental analysis of diopside. Brunauer–Emmett–Teller (BET) method with nitrogen gas adsorption

Download English Version:

<https://daneshyari.com/en/article/7866818>

Download Persian Version:

<https://daneshyari.com/article/7866818>

[Daneshyari.com](https://daneshyari.com)