Materials Science and Engineering C 29 (2009) 1759–1764

Contents lists available at ScienceDirect

Materials Science and Engineering C

journal homepage: www.elsevier.com/locate/msec

Bioactivity of CaO–MgO–SiO_2 glass ceramics synthesized using transferred arc plasma (TAP) process

C.P. Yoganand [a], V. Selvarajan [a,*], Luca Lusvarghi [b], O.M. Goudouri [c], K.M. Paraskevopoulos [c], Mahmoud Rouabhia [d]

[a] Plasma Laboratory, Department of Physics, Bharathiar University, Coimbatore-46, India
[b] Dipartimento di Ingegneria dei Materiali e dell'Ambiente, University of Modena and Reggio Emilia, Via Vignolese, 905, 41100, Modena, Italy
[c] Department of Physics, Aristotle University of Thessaloniki, 54124 Thessaloniki, Greece
[d] Faculté de Médicine Dentaire et Groupe de Recherche en E' cologie Buccale, Pavillon de Médecine Dentaire, Local 1728, Université Laval, Quebec City, Quebec, Canada G1K 7P4

ARTICLE INFO

Article history:
Received 31 October 2008
Received in revised form 6 January 2009
Accepted 26 January 2009
Available online 8 February 2009

Keywords:
Gass ceramic
Diopside
Transferred arc plasma
Simulated body fluid (SBF)
Fibroblast
Bioactivity

ABSTRACT

Glass ceramic with a nominal composition of 35.6% CaO, 12.8% MgO and 51.6% SiO_2 was prepared by transferred arc plasma processing. The in vitro bioactivity of the plasma synthesized CaO–MgO–SiO_2 glass ceramic was examined for its biomedical applicability which was evaluated by immersion in simulated body fluid at 36.5 °C for several days. The apatite particles were found to be formed on the surface of the glass ceramic and grew with the passage of soaking time. The simulated body fluid test results showed the formation of carbonated hydroxyapatite like layer on the surface of the glass ceramic. The cytocompatibility was evaluated through human fibroblast proliferation. The fibroblasts adhere, spread, and proliferate on the CaO–MgO–SiO_2 glass ceramic, and the cell proliferation was more obvious.

1. Introduction

Bioactive glasses and glass–ceramics have been investigated for tissue engineering applications in bone repair and are successfully used in several clinical applications. Unique properties of bioactive glasses are the ability to convert to hydroxyapatite (HAp) in body fluids and in aqueous solutions containing calcium and phosphate ions, and the ability to bond directly to bone. Since its bone bonding properties were reported in 1971 by Hench et al. In the last two decades, remarkable advances in the field of biomaterials have led to the development of bioglasses and bioceramics of various compositions for bone repair and prostheses applications [1–3].

Plasma technology has in recent years emerged as a novel technique for the manufacture of newer and better materials. Plasma technology is an enabling technology, which integrates processes associated with plasma material interaction with manufacturing, adds value to conventional materials and makes new types of materials and material processing techniques possible [4,5]. Previously the authors reported synthesis of glass ceramics by transferred arc plasma (TAP) processing [6]. The TAP melting method is a single step process of preparation of glass–ceramics in which the raw materials are melted in the plasma and crystallization of the melt occurs while quenching.

Good mechanical and chemical properties of glass–ceramic materials in the CaO–MgO–SiO_2 system indicated them as suitable candidate for use in wear resistant, thermo-mechanical, biomedical and ceramic-coating applications [7], furthermore diopside ($CaMgSi_2O_6$), a Ca, Si and Mg-containing ceramic, has been reported by T Nonami and S Tsutsumi to possess apatite formation ability in SBF and can closely bond to bone tissue [8]. In the present investigation, transferred arc plasma synthesized CaO–MgO–SiO_2 glass ceramic was examined for its bioactivity by immersion in simulated body fluid (SBF). The cytocompatibility of synthesized CaO–MgO–SiO_2 glass ceramic was evaluated through human fibroblast proliferation. The bioactivity assessment of TAP synthesized glass ceramic along with its phase and microstructural characterization are presented.

2. Experimental procedure

2.1. Plasma synthesis of CaO–MgO–SiO_2 glass ceramics

The CaO–MgO–SiO_2 glass ceramic was obtained by TAP melting method [6]. Homogeneous mixture of 35.6% CaO, 12.8% MgO and 51.6% SiO_2 (by wt.%) was obtained by dry mixing for 4 h in a ball mill (In smart systems, India). Commercially available 99% CaO, 99% MgO and 99% SiO_2 (quartz) supplied by Sigma-Aldrich was used. After dry mixing, the homogeneous mixture was taken into the anode bed of the dc transferred arc plasma torch. For this experiment, a dc transferred arc plasma torch (Ion Arc Technologies Pvt. Ltd., India)

* Corresponding author. Tel.: +91 422 2422222; fax: +91 422 2422387.
E-mail address: vselvrjn47@rediffmail.com (V. Selvarajan).

0928-4931/$ – see front matter © 2009 Elsevier B.V. All rights reserved.
doi:10.1016/j.msec.2009.01.028

was used for the purpose (Fig. 1). The torch consists of a cylindrical well in a graphite lot, which is the anode (100 mm height and 70 mm outer diameter). The cathode is made of graphite rod 250 mm long and 50 mm diameter. The cathode is enclosed in a hollow brass cylinder and provisions are made for water circulation (for cooling at a flow rate of 12 lpm) and gas flow. The system has multiple inlets for plasma gas at the cathode end. The torch was operated at power level of 5 kW. Argon at a flow rate of 10 lpm was used as the plasma forming gas, Plasma was generated and the mixture was heated for 8 min (i.e. melting time). During which they got melted in the plasma to form glass–ceramic melt. The CaO–MgO–SiO_2 glass ceramic was produced by quenching the melts by applying forced air on it. The operating parameters are given in Table 1. The synthesized glass–ceramic sample was studied for its phase composition and microstructure using X-ray diffraction (PW 3710, Philips with Cu-Kα radiation), energy dispersive spectra analysis and scanning electron microscopy (Philips XL40).

Table 1
Typical operating parameters of TAP torch.

Material & composition	51.6% SiO_2, 35.6% CaO & 12.8% MgO (by wt.%)
Torch type	Transferred arc plasma (TAP) torch
Input power	5 kW
Plasma gas and flow rate	argon; 10 lpm
Cooling water flow rate	12 lpm
Processing time	8 min
Quenching medium	air

2.2. In-vitro bioactivity analysis in SBF

The bioactivity of TAP synthesized glass ceramic was examined by in vitro procedures involving the dissolution in aqueous media, like c-SBF, that was prepared as described by Ohtsuki et al [9]. In the process, 75 mg of CaO–MgO–SiO_2 glass ceramic powder with average particle size of 200 μm was immersed in 50 ml c-SBF at 36.5 °C for several days. Then after filtration, the powders were rinsed with distilled water, dried and a quantity of 0.003 g with 0.2 g of KBr was pressed each time in a vacuum press at 7t in order to produce a pellet with 13 mm diameter and 0.8 mm thickness. The pellets were then characterized with fourier transform infrared spectroscopy (FTIR). The remaining reacted powders were carbon coated in order to be examined with scanning electron microscopy and energy dispersive spectroscopic analysis (SEM–EDX). The FTIR spectra were collected using a Bruker IFS113v FTIR spectrometer, in transmission mode in MIR region (400–4000 cm^{-1}) with a resolution of 4 cm^{-1}.

2.3. Cytocompatibility analysis

Normal human skin fibroblasts were grown in 75 cm^2 cell culture flasks (Falcon, Becton-Dickinson, Cockeysville, MD, USA) in Dulbecco's modified Eagle's medium (DMEM, Invitrogen Life Technologies, Burlington, ON, Canada) with 10% fetal calf serum (FCS, Invitrogen Life Technologies), 100 IU/ml penicillin G, 25 μg/ml streptomycin and 0.5 μg/ml fungizone (Sigma-Aldrich Canada, Ltd.). The cultured cells were then incubated at 37 °C in 98% humidity, and 5% CO_2. When the cultures reached 90% confluence, the cells were detached from the flasks using a 0.05% trypsin-0.1% EDTA solution, washed twice, and used to evaluate their adhesion and growth onto the CaO–MgO–SiO^2 glass ceramic. Fibroblasts were seeded into 24 well plates. Each well contains small pieces of the glass ceramic material. Materials were sterilized using 2×30 mn incubation in 70% ethanol. Following three washes with sterile DMEM, each well was seeded with 103 fibroblasts and cultured for 24 and 48 h. At the end of each incubating period fibroblasts that adhered to the materials were visualized using Hoechst staining. To do so, cells on the materials were fixed with 75% methanol (EMD Chemicals Inc., Gibbstown, NJ, USA) and 25% glacial acetic acid (Laboratoire MAT Inc.) solution (v/v) for 5 mn. Following 2 washes with PBS, each well was then supplemented with 0.5 ml Hoechst dye (1 μg/ml) (Molecular Probes, Eugene, OR, USA) and the specimens were incubated for 15 min at room temperature before being extensively washed with distilled water, observed under

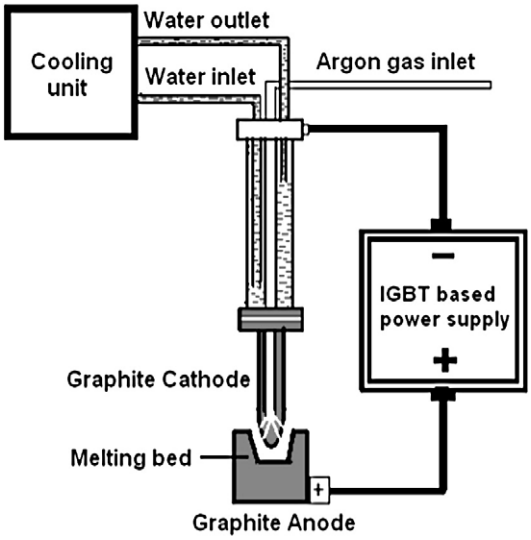

Fig. 1. Schematic drawing of the TAP torch.

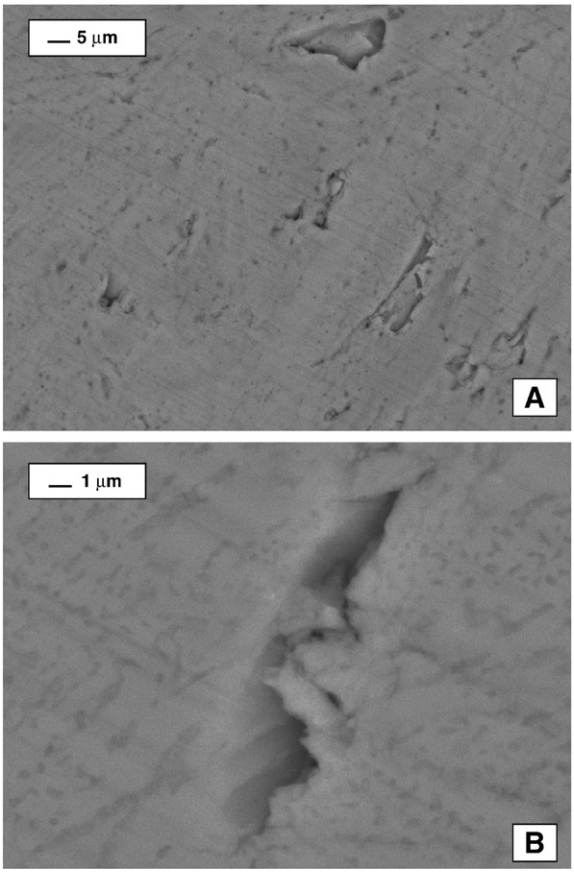

Fig. 2. SEM images at different magnifications of TAP synthesized CaO–MgO–SiO_2 glass ceramic.