Materials Science and Engineering C 73 (2017) 778–787

Contents lists available at ScienceDirect

Materials Science and Engineering C

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

In vitro biocompatibility of a ferrimagnetic glass-ceramic for hyperthermia application

Oana Bretcanu [a,1], Marta Miola [a], Claudia L. Bianchi [b], Ida Marangi [c,2], Roberta Carbone [c,3], Ingrid Corazzari [d,e], Mario Cannas [f], Enrica Verné [a,*]

[a] Applied Science and Technology Department, Institute of Materials Physics and Engineering, Politecnico di Torino, C.so Duca degli Abruzzi 24, 10129 Torino, Italy
[b] Department of Chemistry, University of Milan, Via Golgi 19, 20133 Milan, Italy
[c] Department of Experimental Oncology, European Institute of Oncology, Milan, Italy.
[d] Department of Chemistry, University of Torino, Via Pietro Giuria 7, 10125 Torino, Italy
[e] "G. Scansetti" Interdepartmental Centre for Studies on Asbestos and other Toxic Particulates, Via Pietro Giuria 7, 10125 Torino, Italy
[f] Department of Medical Science, Human Anatomy, University of Eastern Piedmont, Novara, Italy

ARTICLE INFO

Article history:
Received 16 September 2016
Received in revised form 3 November 2016
Accepted 20 December 2016
Available online 23 December 2016

Keywords:
Ferrimagnetic
Glass-ceramic
Magnetite
Cell culture
Hyperthermia
Cancer

ABSTRACT

Ferrimagnetic glass-ceramics containing magnetite crystals were developed for hyperthermia applications of solid neoplastic tissue. The present work is focused on in vitro evaluation of the biocompatibility of these materials, before and after soaking in a simulated body fluid (SBF). X-ray diffraction, scanning electron microscopy, atomic absorption spectrophotometry, X-ray photoelectron spectrometry and pH measurements were employed in glass-ceramic characterisation. The free-radical mediated reactivity of the glass-ceramic was evaluated by Electron Paramagnetic Resonance (EPR) spin trapping. Cell adhesion and proliferation tests were carried out by using 3T3 murine fibroblasts. Cytotoxicity was performed by qualitative evaluation of human bone osteosarcoma cells U2OS cell line. The results show that almost two times more 3T3 cells proliferated on the samples pretreated in SBF, compared with the untreated specimens. Moreover a decrease of confluence was observed at 48 and 72 h for U2OS cells exposed to the untreated glass-ceramic, while the powder suspensions of glass-ceramic pre-treated in SBF did not influence the cell morphology up to 72 h of exposition. The untreated glass-ceramic exhibited Fenton-like reactivity, as well as reactivity towards formate molecule. After pre-treatment with SBF the reactivity towards formate was completely suppressed. The concentration of iron released into the SBF solution was below 0.1 ppm at 37 °C, during one month of soaking. The different in vitro behaviour of the samples before and after SBF treatment has been correlated to the bioactive glass-ceramic surface modifications as detected by morphological, structural and compositional analyses.

1. Introduction

Magnetic materials are widely used for different biomedical applications such as magnetic resonance imaging contrast agents, immunoassay, detoxification of biological fluids, hyperthermia, drug delivery, cell separation, etc. [1,2]. More recently, magnetic materials (nanoparticles) have been included in the category of bio-targeted systems and therapeutic tools [3] which can be guided to target sites by means of an external magnetic field and subsequently heated for hyperthermia cancer therapy. Magnetic nanoparticles are assessed as both carriers for drugs and as contrast agents for magnetic resonance detection. All these applications require appropriate biological behaviour in terms of biocompatibility and non-toxicity. In vitro biological tests have general applicability and widespread use for evaluation of a large range of devices and materials, being a critical factor for biomedical applications.

In the last decades, magnetic glass-ceramics have been developed for the magnetic induction of hyperthermia, which is one of the therapies for cancer treatment [4,5,6,7,8]. This method exploits the potential of magnetic materials to generate heat under an alternating magnetic field. The heat generation depends on the materials properties, magnetic field parameters and tissue characteristics [9]. Magnetic particles are implanted in the tumour site and an external oscillating magnetic field is applied. The magnetic materials will develop heat, raising the temperature of the surrounding tissue and thus, the cancer cells are damaged or destroyed [10].

In our previous studies [11,12,13,14] ferrimagnetic glass-ceramics with the composition in the system SiO_2–Na_2O–CaO–P_2O_5–FeO–Fe_2O_3

* Corresponding author.
E-mail address: enrica.verne@polito.it (E. Verné).
[1] Present affiliation: Newcastle University, School of Mechanical and Systems Engineering, Newcastle upon Tyne, United Kingdom.
[2] Present affiliation: ATS INSUBRIA, Sede Territoriale di Varese, Via O. Rossi, 9, 21,100 Varese, Italy.
[3] Present affiliation: Tethis S.p.A Genextra Group, Via Russoli, 3, 20,143 Milano, Italy.

http://dx.doi.org/10.1016/j.msec.2016.12.105
0928-4931/

were obtained by two different synthesis methods: coprecipitation derived and traditional-melting derived. The microstructure, thermal and magnetic properties, bioactivity and specific power loss were analysed. Focusing the attention on the bioactivity mechanism of glasses and glass-ceramics [15], it was shown that it starts with a rapid ions exchange between the alkaline ions from the glass surface and the hydrogen ions from the solution. This step is followed by the formation of silanols on the surface of the glass, which then undergo polycondensation to form a silica gel layer. Silica gel promotes the adsorption of calcium and phosphate ions from the solution, which subsequently react, forming a hydroxyapatite layer.

The first stages of bioactivity, up to the formation of silanols on the glass surface, give rise to a slight enhancement of pH values due to the depletion of the hydrogen ions in the solution. This feature can be also observed in solutions such as TRIS buffer or Kokubo's simulated body fluid (SBF) [16] and should be carefully controlled when bioactive glass samples are placed in contact with different cell lines, during biological evaluations [17].

If iron oxides are present into the glass or glass-ceramic, some effort should be done to assess that ion leaching can occur without production of toxic ions in vivo, taking into account that, however, the body has a mechanism for the management of Fe^{3+}, so its low-level release should not produce any toxic effects [18]. Moreover, when particles come in contact with cells and tissues, the interactions between particles surface and the living matter can have detrimental outcomes. As observed with toxic particulates including lunar dust simulants and volcanic ashes [19, 20], the presence of redox active centres (more likely iron) is responsible for the oxidative potential of such particulates. In particular, they can drive the release of reactive oxygen species (ROS), such as hydroxyl, superoxide and hydroperoxide radicals. A mechanism through which iron (or other redox-active metals) centres are implied in the generation of HO• radical is the Fenton-like reaction which occurs in the presence of H_2O_2, as in the phagolysosomal vesicles after phagocytosis. A complementary reaction driven by redox active centres is the homolytic cleavage of the C—H bond of biomolecules (including lipids, proteins and nucleic acids).

In this paper we focus on the surface reactivity and ion leaching of ferrimagnetic glass-ceramics in the system SiO_2–Na_2O–CaO–P_2O_5–FeO–Fe_2O_3, and on their biological properties by analysing the in-vitro biocompatibility on 3T3 murine fibroblasts and U2OS human bone osteosarcoma osteoblasts. Moreover, the capability of SC45 to generate HO• radicals via a Fenton-like reaction and to cleave the C—H bond of formate ion was investigated by electron paramagnetic resonance (EPR) spin trapping.

2. Materials and methods

2.1. Sample preparation

A ferrimagnetic glass-ceramic with the nominal composition 24.7SiO_2–13.5Na_2O–13.5CaO–3.3P_2O_5–14FeO–31Fe_2O_3 (wt.%) was obtained by a traditional melting method, quenching the melt from 1550 °C melting temperature onto a rectangular brass mould as described in [12]. The resulted glass-ceramic, named SC45, contains magnetite crystals, embedded in an amorphous matrix. Magnetite crystals are formed during quenching. The obtained bars were annealed at 600 °C for 12 h and then were polished with SiC abrasive papers, up to 2400 grit. A part of the bars was cut in small slices (around $5 \times 5 \times 2 mm^3$). Another part was ground in a planetary ball mill and the obtained powder was sieved under 20 μm.

2.2. Characterisation of the ferrimagnetic glass-ceramic

2.2.1. X-ray diffraction (XRD)

X-ray diffraction (XRD) patterns were registered by a Philips X'Pert diffractometer with Cu K_α radiation, between 10° and 75° (2θ), at 40 kV and 30 mA. The phase identification was performed by X'Pert HighScore program, with PCPDFWIN database.

2.2.2. Scanning electron microscopy (SEM)

The glass-ceramic morphology was analysed by scanning electron microscopy (SEM), using a FEI, QUANTA INSPECT 200 model. In order to analyse the crystals morphology, the polished glass-ceramic bulk samples were first etched for 4 min with a solution of 5% volume of HF:HNO_3 in a molar ratio 1:1, and then coated by a thin layer of silver.

2.2.3. pH analysis

The variation of the pH during soaking the samples in 15 ml SBF, prepared after Kokubo's protocol [21] over one month period, was measured by a pocket sized pH-meter (Hanna Instruments). Both bulk and powder samples were used. The bulk samples had dimensions around $5 \times 5 \times 2 mm^3$, while the powders (0.2 g/sample) had the particles size <20 μm. The samples were kept at 37 °C in an incubator. The SBF solutions were refreshed twice a week for both powders and bulk samples. For the bulk samples, the pH was measured without refreshing the SBF solution. Four samples were used for each set of experiments and the average pH value was calculated.

2.2.4. Atomic absorption spectrophotometry (AAS)

Samples containing 0.2 g of SC45 powder were immersed in 15 ml SBF solution and respectively distilled water, and kept at 37 °C in an incubator for a maximum of four weeks. The variation of the concentration of Ca, Si, Na and Fe released during soaking the SC45 powder samples in both distilled water and SBF was measured by a Perkin-Elmer 1100B Atomic Absorption Spectrometer equipped with an air-acetylene flame burner. Each chemical element was measured individually. The experiments were performed in triplicate.

2.2.5. X-ray photoelectron spectroscopy (XPS)

X-Ray photoelectron spectroscopy (XPS) spectra were registered on a Surface Science Instrument (M-Probe) with a monochromatic Al Kα X-ray source. The detailed spectra of oxygen, carbon and silicon regions (O1s, C1s and respectively Si2p) were analysed separately. The take-off angle to the substrates was 90°, the pressure was approximative 10^{-8} Torr and the pass energy used to determine the elemental composition was 30 eV. Two sets of bulk samples were analysed: SC45 samples before and after soaking in SBF for one week (samples denominated SC45 + SBF).

2.2.6. Electron paramagnetic resonance (EPR)/spin trapping

The generation of free radicals was monitored by electron paramagnetic resonance (EPR) spectroscopy using a Miniscope MS 100 (Magnettech, Berlin, Germany) spectrometer. 5,5-Dimethyl-1-Pyrroline-N-Oxide (DMPO) was employed as the spin trapping agent. The instrument settings were the following: microwave power 10 mW, modulation 1000 mG, scan range 120 G and the centre of field approximately 3345 G.

2.2.6.1. Surface-driven Fenton reactivity (target molecule H_2O_2)

SC45 was suspended in a H_2O_2 (0.04 M) buffered solution (potassium phosphate buffer, KPB 125 mM, pH 7.4) at the dose of 45 mg/ml in the presence of DMPO (0.075 M) as spin-trapping agent. During the experiment, the suspension was continuously stirred. 50 μl of the suspension were withdrawn after 10, 30 and 60 min of incubation and the EPR spectra were recorded. The experiments were performed in triplicate. In order to assess the persistence of the reactivity of SC45 after prolonged contact with biofluids, the same experiments were carried out on SC45 previously incubated with SBF for one week (SC45 + SBF samples). Blanks experiments were performed in parallel in the absence of SC45 powders.