Colloids and Surfaces B: Biointerfaces 148 (2016) 592–599

Contents lists available at ScienceDirect

Colloids and Surfaces B: Biointerfaces

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

Gallic acid grafting modulates the oxidative potential of ferrimagnetic bioactive glass-ceramic SC-45

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ARTICLE INFO

Article history:
Received 5 May 2016
Received in revised form 22 July 2016
Accepted 23 September 2016
Available online 23 September 2016

Keywords:
Ferrimagnetic glass-ceramic
Gallic acid
Free radical release
Lipoperoxidation
Bioactivity
Iron reduction

ABSTRACT

Magnetite-containing glass-ceramics are promising bio-materials for replacing bone tissue after tumour resection. Thanks to their ferrimagnetic properties, they generate heat when subjected to an alternated magnetic field. In virtue of this they can be employed for the hyperthermic treatment of cancer. Moreover, grafting anti-cancer drugs onto their surface produces specific anti-neoplastic activity in these biomaterials. Gallic acid (GA) exhibits antiproliferative activity which renders it a promising candidate for anticancer applications. In the present paper, the reactivity of ferrimagnetic glass-ceramic SC-45 grafted with GA (SC-45+GA) was studied in terms of ROS release, rupture of the C–H bond of the formate molecule and Fenton reactivity by EPR/spin trapping in acellular systems. The ability of these materials to cause lipid peroxidation was assessed by UV–vis/TBA assay employing linoleic acid as a model of membrane lipid. The results, compared to those obtained with SC-45, showed that GA grafting (i) significantly enhanced the Fenton reactivity and (ii) restored the former reactivity of SC-45 towards both the C–H bond and linoleic acid which had been completely suppressed by prolonged contact with water. Fe^{2+} centres at the surface are probably implicated. GA, acting as a pro-oxidant, reduces Fe^{3+} to Fe^{2+} by maintaining a supply of Fe^{2+} at the surface of SC-45+GA.

1. Introduction

Bioactive glasses and glass-ceramics are versatile materials for bone-tissue replacement. The properties of such materials, depending upon their composition, can be tailored to specific needs. By varying the glass composition (mainly SiO_2 and P_2O_5 content), it is possible to obtain quite stable as well as highly bioactive glasses and even fully resorbable materials [1–4]. Moreover, the introduction of different metal oxides allows the material reactivity to be further modulated and the release of ions with specific biological activities, including the stimulation of osteoblast proliferation, bone formation and mineralization, and antibacterial activity, to be induced [3–6]. Thanks to their particular structure, specific ions (*e.g.* Ag^+ for antibacterial properties) can be put on the surface by means of an ion exchange process [4,7].

Besides their applications in producing scaffolds, bone fillers and coatings, bioactive glasses and glass-ceramics have been exploited to repair bone defects after tumour resection [8]. In particular ferrimagnetic glass-ceramics find their principal scope in contributing to bone healing after cancer removal. Ferrimagnetic properties are assured by the presence of magnetite crystals (Fe_3O_4) embedded in the glass matrix [9]. Magnetite crystals nucleation is obtained by introducing iron oxides into the glass composition [9–13]. It is well known that under an alternating magnetic field, magnetic materials can generate heat by hysteresis loss. An increasing interest on hyperthermia has been shown by the scientific community due to its promising medical applications. Hyperthermia can be considered as a specific anticancer treatment, since cancer cells tolerate heat less efficiently than healthy cells when the temperature of the treated tissue reaches 44 °C [14]. Moreover several authors are developing drug-delivery systems where the hyperthermic effect, assured by the presence of magnetic particles, can be exploited to induce the release of specific drugs embedded in a

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http://dx.doi.org/10.1016/j.colsurfb.2016.09.034
0927-7765/

polymeric matrix [15,16]. When the ferrimagnetic glass-ceramic is subjected to an alternating magnetic field, heat is generated causing a local rise of the temperature (42–45 °C). The induced hyperthermia can damage directly tumour cells or make them more susceptible to radio/chemotherapy. For this reason, it can be exploited as a complementary therapy in the treatment of bone cancer besides surgery, radio- and/or chemo-therapy. In the case of bone implants after tumour resection, this local overheating can act preferentially against new bone-tumour formation after the surgical removal in case of relapse. Moreover, a recent study by some of the authors refers a positive influence of the mild magnetic field induced by magnetite crystals on cell viability [17], an effect that is not provided by superparamagnetic nanoparticles, which do not retain any magnetism after removal of the external magnetic field.

After implantation, bioactive glasses and glass-ceramics interact with bone cells, bio-fluids and biomolecules, causing ion-exchange reactions culminating with the precipitation of calcium phosphates and the crystallization of hydroxyapatite on the biomaterial surface [1]. The formation of a hydroxyapatite layer induces the chemical bonding between the artificial surface and the bone tissue and thus it represents a beneficial outcome of the interaction with biofluids. For this particular application, in addition to the general features required of bone substitute materials (*i.e.* biocompatibility, bioactivity, bio-stability, and bio-degradability) [18], a specific anti-neoplastic activity is desirable. The exploitation of bioactive glasses and glass-ceramics functionalized with chemotherapics (*e.g.* doxorubicin, *cis*-platinum complexes or dexamethasone) for the local treatment of bone cancer results in an increased efficiency of the therapy minimizing the undesired effects due to the systemic administration of anti-neoplastic drugs [19,20]. Grafting bio-active molecules onto the surface of ferrimagnetic glass-ceramics may be an interesting strategy to obtain specific therapeutic tools which combine hyperthermia with the anti-cancer properties of bioactive molecules. Some studies documented selective toxicity of polyphenols towards cancer cells [21] and their ability to stimulate healthy osteoblasts [22]. Moreover, polyphenols exert, on one hand, a protective role on healthy cells and, on the other, a sensitizing effect on tumour ones rendering them more susceptible to antineoplastic drugs [23,24]. These molecules exhibit also a certain thermal stability which preserves their activity in the temperature range of cement hardening and hyperthermia treatment [25]. In previous works, some of the authors modified the surface of the well characterized ferrimagnetic glass-ceramic SC-45 with gallic acid (3,4,5-trihydroxybenzoic acid, GA) [26]. GA is a phenolic acid widely distributed in many different families of higher plants. In nature it exists both in free state, and as a precursor of polyphenols. It was chosen because, besides its recognized antioxidant activity, it shows some specific anti-tumour properties. In particular, the literature reports that GA can induce apoptosis of the tumour cells *via* different mechanisms depending upon the cell type, but all these mechanisms are likely driven by ROS generation [27]. Moreover, some authors have observed that GA strongly inhibits cancer cell migration and metastasis by suppressing the expression of some metalloproteinases [28]. The presence of GA could modify the surface reactivity of a magnetite-containing biomaterial acting as a reducing agent for iron (III). As observed with toxic particulates including lunar dust simulants and volcanic ashes [29,30] the presence of redox active centres (most likely iron) accounts for the oxidative potential of these particulates. Analogously, SC-45 could show a similar reactivity mediated by iron centres at the surface of the magnetite crystals. Moreover, the interaction of redox active metal ions and some antioxidant molecules enhances the reactivity mediated by iron [29,31]. In the present article the chemical interactions of SC-45 modified with GA and some models of biologically relevant molecules, mimicking the environment this biomaterial may be in contact with after implantation, has been considered.

Table 1
Chemical composition (as oxides%) of SC-45 glass-ceramic.

	Fe_2O_3	FeO	SiO_2	CaO	Na_2O	P_2O_5
wt.%	31	14	24.7	13.5	13.5	3.3

In particular, the anti-oxidant/pro-oxidant potential of the bare glass-ceramic compared to the gallic acid-grafted one has been investigated for the first time. Since SC-45 is a ferrimagnetic glass-ceramic with heating ability under alternating magnetic field, it can be applied for bone substitution after cancer removal. GA grafting on its surface can potentiate its activity in cancer treatment. In this context surface reactivity was investigated because it can play a crucial role in exerting toxic outcomes with a dual effect representing a useful tool which can be exploited to kill tumour cells, but also a possible interference with the process of bone regeneration.

2. Material and methods

2.1. Reagents

Where not otherwise specified, all the reagents were from Sigma Chemicals (Sigma-Aldrich St. Louis, MO, USA), and all the solutions employed were prepared with Milli-Q ultrapure water system (Merck Millipore, Darmstadt, Germany).

2.2. Material preparation

SC-45 glass-ceramic was prepared by traditional melt and quenching technique and fully characterized in previous works [13,20,32]. Its composition (wt%) is reported in Table 1.

The obtained glass-ceramic was ball milled and sieved up to a grain size of 20 μm.

The powder surface was functionalized with gallic acid via direct grafting to the surface exposed OH groups without any spacer, as described in Ferraris et al. [26].

Briefly SC-45 powder was suspended in ultrapure water (0.10 g powder in 10 ml water) for one week at 37 °C in order to expose –OH groups at the surface, as described in Vernè et al. [20]. Hydroxyl groups are fundamental for the direct grafting of gallic acid molecules on the glass-ceramic surface. After incubation, the powder was separated from the supernatant with the use of a magnet, and dried under a laminar flow cabinet (FASTER CYTOSAFE) in order to avoid surface contaminations. The dried powder (hereafter named SC-45-pretreated) was suspended in an aqueous solution 1 mg/ml of GA (0.1 g powder in 5 ml of GA solution) for 3 h at 37 °C. After incubation, the sample was separated from the supernatant by the use of a magnet, gently washed twice in ultrapure water and dried under the laminar flow cabinet. The GA functionalized sample is hereafter named SC-45+GA. A thorough physico-chemical characterization of the SC-45+GA was object of a previous study [26].

2.3. Bioactivity

In order to evaluate *in vitro* the bioactivity of SC-45 samples before and after gallic acid grafting, the powders were soaked in Simulated Body Fluid (SBF) [33] for 14 and 28 days (0.1 g powder in 25 ml SBF) at 37 °C in an incubator. Solution refresh was performed every two days in order to mimic the physiological turnover of body fluids. At each refresh the solution pH was measured. SBF was prepared according to the protocol proposed by Kokubo [34].

At the end of the soaking period samples were gently washed in ultrapure water and dried under a laminar flow cabinet. In order to investigate surface reactivity and hydroxyapatite precipitation, samples were deposited onto conductive carbon tape, fixed to alu-