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# Gallic acid grafting to a ferrimagnetic bioactive glass-ceramic

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ABSTRACT

Ferrimagnetic bioactive glass ceramics are promising biomaterials in the field of bone substitution and cancer treatment for their ability to bond to bone (bioactive behavior) and to be heated by the application of an external magnetic field (hyperthermia). Surface functionalization of these materials with polyphenols is a challenging and innovative strategy in order to impart them additional functional and specific properties (e.g. antioxidant, anticancer and antibacterial). Gallic acid (GA) is a phenolic acid which can be considered a good model molecule for polyphenols due to its simple structure and representative properties. In the present paper GA has been grafted to a ferrimagnetic glass ceramic (SC-45), in bulk and powder forms, in view of its potential clinical applications (such as hyperthermic treatment of cancer combined with the anticancer action of GA). The grafting process has been optimized in order to preserve GA activity. The effectiveness of the functionalization procedure has been demonstrated by means of Scanning Electron Microscopy equipped with Energy Dispersive Spectroscopy (SEM-EDS), X-ray Photoelectron Spectroscopy (XPS), Thermogravimetric analysis-gas evolved analysis (TGA-EGA) and Folin&Ciocalteu tests (F&C). Release tests have been performed in double distilled water at 37 °C and 43 °C to verify the stability of the material.



## 1. Introduction

Bioactive glasses and glass-ceramics are particular biomaterials able to allow the growth of hydroxyapatite layer on their surface. After implantation, these materials react with physiological fluids by means of a series of ion exchange reactions that culminate with the precipitation of calcium phosphates and the crystallization of hydroxyapatite on the biomaterial surface [1,2]. In the biological environment a series of biological reactions (absorption of biological molecules, action of macrophages, attachment and differentiation of stem cells, osteoblast proliferation, generation and crystallization of matrix) follow the inorganic ones and lead to new bone formation [1,2]. The structure of these materials makes possible to obtain many different compositions with peculiar and tailored properties. Varying the silica content it is possible to modulate the glass bioactivity from almost inert to highly bioactive and substituting $SiO_2$ with $P_2O_5$ it is possible to obtain fully resorbable structures [3]. The introduction of different modifying oxides allows the release of ions with specific stimulation ability for cells: $Ca^{++}$ for osteoblast proliferation, differentiation, and mineralization, $Mg^{++}$ for cellular adhesion and bone formation, $Zn^{++}$, $Cu^{++}$, and $Ag^{+}$ for an antibacterial action, to cite only some examples [4,5]. The effect of different ions on the bone bonding and antibacterial properties of bioactive glasses have recently been reviewed [6,7]. It has been observed that bioactive glasses and their dissolution products can stimulate bone regeneration by affecting some cellular functions at the genetic level [8].

Thanks to their ability to induce the formation of a hydroxyapatite layer and to stimulate the formation of new bone tissues bioactive glasses and glass-ceramics have been historically intended for orthopedic and dental applications. In the last years their ability to bond to soft tissues, to act as carriers for drugs and bioactive molecules, to exploit specific functional properties based on their composition and crystalline phases suggested the possibility of their application in different fields such as soft tissue regeneration, wound healing, cancer therapy and local delivery [9,10].

The authors previously developed a ferrimagnetic glass-ceramic (SC-45) [11–13] characterized by magnetite crystals embedded in an amorphous bioactive matrix (analogous to Bioglass®). The presence of magnetic crystals makes possible to heat the material by the application of an external magnetic field. This peculiar feature allows the application of this glass-ceramic for hyperthermia in cancer treatment. Moreover in a previous research work the grafting of chemotherapeutic drugs has been considered in order to couple hyperthermic therapy with pharmacological one [13]. More recently, powders of SC-45 glass-ceramic have been used as dispersed phase to develop a new composite PMMA-based bone cement with bioactive and ferrimagnetic properties [14].

Polyphenols are a class of natural molecules, present in many plants and vegetable-based foods and beverages. An increasing interest in

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their study can be registered in the last years mainly due to the potential health benefits associated with these substances, such as anti-oxidant, anti-carcinogenic, antibacterial, anti-inflammatory, cardio- and neuro-protective effects [15–18]. An inhibitory effect of various polyphenols (including gallic acid) on molecular mechanisms associated to chronic inflammation, tumor genesis, progression, invasion and metastasis, have been documented in vitro and in vivo [19–21]. Gallic acid (3,4,5-trihydroxybenzoic acid) is a simple and small molecule belonging to phenolic acids and it can be considered representative for this class of molecules. A pro-apoptotic effect of gallic acid has been evidenced against several cancer cell lines, such as lung cancer cells [22], HeLa cervical cancer cells [23], prostate carcinoma DU145 cells [24] and oral cancer cells [25]. A preferential pro-apoptotic effect of gallic acid against cancer cells compared to healthy ones, has been observed in vitro [23,25]. The ability of this molecule to inhibit the growth and progression of prostate cancer after oral administration has been observed in a mouse model [26].

Despite of a wide research on the effects of the pure molecule on in vitro and in vivo models, few attempts of its coupling with artificial carriers have been reported. Combination of gallic acid with chitosan [27–29], dendrimers [30], Mg/Al layered double hydroxide [31], magnetite and gold nanoparticles [32,33] can be cited as examples.

In a previous paper [34] the authors reported the possibility to effectively graft gallic acid to bioactive surface of glasses with different degree of bioactivity. In the present research work, for the first time, the authors report gallic acid grafting to a ferrimagnetic bioactive glass-ceramic. The presence of a crystalline phase (magnetite) and of iron ions affect both the application of the modified materials (specifically intended for cancer treatment) and also the physical–chemical characteristic of the surface and the consequent results of the functionalization process.

This paper aims to functionalize with gallic acid (GA) and characterize a bioactive and ferrimagnetic glass-ceramic in view of its potential clinical applications, as that of hyperthermic treatment of cancer combined with the anticancer action of GA. The surface grafting of GA to SC-45 glass ceramic (in bulk and powder forms) has been optimized. The modified surfaces have been characterized from the morphological and chemical points of view, by way of Scanning Electron Microscopy, X-ray Photoelectron Spectroscopy, Folin&Ciocalteu test and Thermogravimetric analyses, in order to determine the presence and redox reactivity of GA. Release tests have also been performed in order to evaluate the eventual molecular release. The antioxidant/pro-oxidant activity and selective cytotoxic behavior against cancer cells will be investigated and reported in future papers.

## 2. Materials and methods

### 2.1. SC-45 bioactive and ferrimagnetic glass-ceramic preparation

The bioactive and ferrimagnetic glass ceramic (SC-45), with the composition (wt.%) 24.7 $SiO_2$, 13.5 $Na_2O$, 13.5 CaO, 3.3 $P_2O_5$, 14 FeO, and 31 $Fe_2O_3$, was prepared by traditional melt and quenching technique, as reported in [11–13]. In brief, commercial reagents ($Na_2CO_3$, $CaCO_3$, $SiO_2$, $Ca_3(PO_4)_2$, $FeSO_4{*}7H_2O$ and $Fe_2O_3$, >99.0%, Sigma Aldrich) were melted in a platinum crucible for 30 min at 1550 °C and then poured on a brass plate. The heating rate applied in order to reach 1550 °C was 10 °C/min. A part of the obtained glass ceramic was annealed 12 h at 600 °C and then cut and polished for obtaining bulk samples (SC-45 bulk). Another part was ball milled and sieved up in order to obtain powders with a grain size below 20 μm (SC-45 pow).

### 2.2. Surface activation and functionalization

In order to expose surface reactive hydroxyl groups (–OH) a water based pretreatment, previously optimized [13] for this specific glass-ceramic was applied. Each SC-45 sample, both bulk (slices 0.38 ± 0.07 g in weight and 98 ± 3 $mm^2$ of exposed area) or powders (0.10 g, 1.8 $m^2/g$) were soaked in 10 ml of double distilled water at 37 °C for 1 week. At the end of the soaking period, the samples were let dry under a laminar flow cabinet (FASTER CYTOSAFE) in order to avoid surface contamination.

Various experimental conditions were considered for GA grafting, in order to find the optimal conditions able to preserve the biomolecule activity and to obtain an effective interaction with the glass-ceramic:

- GA grafting for 24 h at 37 °C (GA 24 h@37 °C),
- GA grafting for 24 h at 37 °C in citric acid–sodium citrate buffer (GA + buf 24 h@37 °C),
- GA grafting for 3 h at 37 °C (GA 3 h@37 °C),
- GA grafting for 3 h at 37 °C with citric acid addition (GA + CA 3 h@37 °C),
- GA grafting for 24 h at 37 °C with citric acid addition (GA + CA 24 h@37 °C).

For all the experiments a 1 mg/ml solution of gallic acid (GA 97.5–102.5% titration, G7384, Sigma Aldrich) was employed, as described in [34,35]. Except for the citric acid–sodium citrate buffer case (GA + buf 24 h@37 °C), GA was dissolved in double distilled water. In the (GA + buf 24 h@37 °C) case GA was dissolved in sodium citrate buffer at pH 3.0. Citric acid–sodium citrate buffer was prepared mixing 82 ml of 0.1 M citric acid and 18 ml of 0.1 M sodium citrate, as described in [36]. The addition of citric acid in experimental setups (GA + CA 3 h@37 °C) and (GA + CA 24 h@37 °C) was performed drop by drop in the aqueous solution of GA up to pH 3.0.

The employment of citric acid–sodium citrate buffer and the addition of citric acid were considered in order to avoid an excessive pH increase of GA solutions during functionalization and avoid its degradation [37,38]. In fact, the glass ceramic can induce a significant increase in the solution pH, due to ionic exchange, during the functionalization period [13].

At the end of the functionalization period, samples (both bulks and powders) were gently washed two times with double distilled water and let dry under a laminar flow cabinet.

All the containers for samples functionalization and storage were covered with aluminum foils in order to avoid light induced degradation of GA.

The pH of the functionalization solutions was measured at different steps of the treatment and their colors registered in order to evaluate possible alterations of GA.

### 2.3. Surface characterizations

The surface morphology and semi-quantitative chemical composition of bulk samples were investigated by Scanning Electron Microscopy equipped with Energy Dispersive Spectroscopy (SEM-EDS, SEM-FEI, Quanta Inspect 200, EDS-EDAX PV 9900), after sputter coating of the samples with Cr.

The chemical composition of the outermost surface layer was investigated by means of X-ray Photoelectron Spectroscopy (XPS, PHI 5000 VERSAPROBE, PHYSICAL ELECTRONICS) on bulk samples. Both survey spectra and high resolution spectra of carbon and oxygen regions were acquired in order to evaluate GA presence on functionalized samples.

### 2.4. Folin & Ciocalteu quantification of GA in solutions and on samples surface

The Folin&Ciocalteu (F&C) method [39] was employed for the quantification of GA both in the functionalization solutions and on sample surfaces. The method, widely employed for the total phenol quantification, determines the GA content by monitoring the reaction between GA, in the sample, and phosphotungstic/phophomolybdic acids, contained in the F&C reagent [34,39]. The principle of the test is based on the reduction ability of GA and the test signal is a consequence of a redox reaction of

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