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Surface functionalization of bioactive glasses with natural molecules of biological significance, Part I: Gallic acid as model molecule

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ABSTRACT

Gallic acid (3,4,5-trihydroxybenzoic acid, GA) and its derivatives are a group of biomolecules (polyphenols) obtained from plants. They have effects which are potentially beneficial to heath, for example they are antioxidant, anticarcinogenic and antibacterial, as recently investigated in many fields such as medicine, food and plant sciences. The main drawbacks of these molecules are both low stability and bioavailability. In this research work the opportunity to graft GA to bioactive glasses is investigated, in order to deliver the undamaged biological molecule into the body, using the biomaterial surfaces as a localized carrier. GA was considered for functionalization since it is a good model molecule for polyphenols and presents several interesting biological activities, like antibacterial, antioxidant and anticarcinogenic properties. Two different silica based bioactive glasses (SCNA and CEL2), with different reactivity, were employed as substrates. UV photometry combined with the Folin&Ciocalteu reagent was adopted to test the concentration of GA in uptake solution after functionalization. This test verified how much GA consumption occurred with surface modification and it was also used on solid samples to test the presence of GA on functionalized glasses. XPS and SEM-EDS techniques were employed to characterize the modification of material surface properties and functional group composition before and after functionalization.

1. Introduction

Gallic acid (3,4,5-trihydroxybenzoic acid, henceforth GA) and its derivatives are present in many plants and are common components of food and beverages of plant origin, such as grape and tea [1]. GA can be extracted from wood, fruits, wine, different alcoholic beverages and various medicinal plants often used in traditional Chinese medicine [2]. Singh et al. isolated various antioxidant phytochemicals from tomatoes, including GA [3]. In Owen's [4] research, carbon fiber was taken as the raw material to extract several classes of polyphenolic substances with the organic solvent extraction, a number of techniques were employed for their analysis: liquid-chromatography electrospray-ionization mass spectrometry (LC–ESI), nano-electrospray-ionization mass spectrometry (ESI–MS) and gas-chromatography mass spectrometry (GC–MS); a total of 24 polyphenol compounds were identified and the amount of GA was 42% of the total phenol mass.

Because of its good biological and pharmacological abilities, it has antioxidant, anti-allergic, anti-carcinogenic, anti-mutagenic and antibacterial properties, GA has been widely used in food additives and cosmetics. In addition, GA is employed as a source material for inks, paints and color developers [5]. Agarwal et al. reported that GA is a major active agent in grape seed extract and induces apoptosis activity in DU145 human prostate cancer cells [6]. GA can also scavenge free radicals of L1210 leukemia cells, which could cause an aberrance of normal tissue [7]. On the other hand some authors reported the pro-oxidant and cytotoxic effect of gallic acid in certain conditions [8–10]. Moreover it has been evidenced that GA coupling with gold nanoparticles can reduce its toxicity [11].

Nowadays, much attention has been paid to the combination of biomaterials and biological molecules. Surface functionalization of biomaterials with biomolecules is a promising strategy in order to target specific signals to cells and tissues directly from material surfaces.

Many research papers dealing with the surface grafting of proteins, enzymes and drugs can be listed [12–16] but few consider polyphenols and natural biomolecules. However, there is an increasing interest for this kind of substances within the scientific community. For instance, GA has been successfully employed as an intermediate functional molecule for further immobilization of vascular endothelial growth factor (VEGF) [17]. Furthermore, in addition to the study and use of the pure molecule, GA has also been combined with organic and inorganic carriers in order to take

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advantages of its antioxidant, antibacterial and anticancer properties.

As far as organic carriers are concerned, recently the coupling of GA with chitosan has been investigated by a number of research groups in order to exploit GA antioxidant properties in the food industry and in pharmaceutical and medical applications [18–20].

Regarding inorganic carriers, GA has been intercalated in Mg/Al layered double hydroxide by co-precipitation [21] to obtain a gradual release of the antioxidant molecule in physiological media.

Finally, the coupling of GA with inorganic nanoparticles has been explored. In particular, magnetite nanoparticles coated with a GA layer have been synthesized for the controlled release of GA in cancer treatment [22]. Moreover, GA has been used to induce significant antibacterial properties and to act as stabilizing agent with gold nanoparticles [23].

Scientific studies on the surface functionalization of synthetic supports with biological molecules and drugs are mainly related to polymers, metals and ceramics and only a few papers have been published on the modification of bioactive glasses.

Bioactive glasses and glass–ceramics are very attractive materials, widely investigated for many years and mainly used in the field of bone reconstruction [24]. They are known for their peculiar surface reactivity when soaked in water or aqueous solutions, like simulated body fluids (SBF), TRIS buffer or physiological media [24–28]. When soaked in simulated or biological fluids, these glasses are prone to various surface modifications which stimulate the precipitation of a hydroxyapatite layer on them. This feature is related to their ability, in vivo, of promoting effective osteointegration [29].

As well-documented by the literature [30], the bioactivity mechanism starts with a rapid ion exchange at the glass surface, between the alkaline ions of the glass and the hydrogen ions of the solution. This causes the formation of silanols that, by further polycondensation, develop a silica gel layer on top of the glass. In this layer the adsorption of calcium and protonated forms of phosphate ions is promoted [27], leading to the crystallization of hydroxyapatite. Due to their hydroxylation properties, bioactive glasses can be successfully functionalized, without further surface activation treatments, if the fast condensation of silanols to silica gel is prevented and thus the presence of free –OH groups on their surface is preserved.

In this research, two bioactive glasses with different degree of bioactivity were chosen as model surfaces for grafting with GA. SCNA is a glass characterized by a simple composition and high stability while CEL2 is a highly bioactive glass [31]. These two biomaterials were previously developed and fully characterized by the authors; their different degree of surface reactivity in simulated body fluids allowed the optimization of their surface activation and the understanding of the role of exposed surface groups on grafting with several biomolecules, like carnosine, bone morphogenetic dipeptide and alkaline phosphatase [32,33]. Bioactive glasses have been chosen as model surface because the combination of their peculiar properties, such as biocompatibility, bioactivity, and tailorable surface reactivity, with the biological properties of GA, represents a challenging approach to the development of implants with improved therapeutic performances. Moreover synthetic supports like glasses are intended to improve the stability and bioavailability of GA at the desired site.

2. Materials and methods

2.1. Samples preparation

Two bioactive glasses (SCNA and CEL2), with different degree of bioactivity, were considered as substrates for GA grafting. The glasses were produced by traditional melt and quenching techniques, as described in several previous papers [31–33]. Composition and melting conditions for the both materials are reported in Table 1.

Table 1
Glasses molar composition and melting/annealing conditions.

	SCNA	CEL2
	Composition [Mol%]	
SiO_2	57	45
P_2O_5	–	3
CaO	34	26
MgO	–	7
Na_2O	6	15
K_2O	–	4
Al_2O_3	3	–
Melting	1 h, 1550 °C	1 h, 1400 °C
Annealing	10 h, 600 °C	12 h, 500 °C

All materials were used both in the bulk and in the powder form.

The bulk materials were obtained by pouring the melted glass on a brass plate. The obtained bars were annealed in a furnace (annealing condition are reported in Table 1) in order to release the residual stresses and cut with an automatic cutter (Struers Accutom 5). Glass slices were then polished with SiC abrasive papers (120–4000 grit) in order to obtain plane and homogeneous surfaces.

For the preparation of powders, the melted glasses were poured in water in order to obtain a frit. The frit was ball milled and sieved up to a grain size low than 20 μm.

2.2. Surface activation

In order to graft GA on the material surface it is necessary to have free –OH groups suitable for the functionalization.

As far as silica-based glasses and glass–ceramics are concerned, it is possible to effectively expose reactive hydroxyls by means of simple water washings and this process was optimized by the authors in previous works [32,33].

The activation step includes a 5 min. washing treatment in acetone in an ultrasonic bath, in order to remove surface contaminants, and 3 subsequent water washing steps (5 min each) in an ultrasonic bath for –OH groups exposition [32]. At the end of the surface activation, samples were dried in air at room temperature. The surface activated samples will be named glass-washed.

2.3. GA grafting

A stock solution of 1.0 mg/ml of GA in double distilled water was prepared by dissolving GA (GA 97.5–102.5% titration, G7384, Sigma Aldrich) in double distilled water for about 2 h under magnetic stirring. For each sample 5 ml of 1.0 mg/ml GA solution was employed. Activated samples (both bulk and powder ones) were soaked in GA solution for 24 h at 37 °C. In order to prevent the light irradiation of GA (which is a light-sensitive molecule) all the holders were covered with aluminum foils.

The effect of pH on the glass and molecular behavior was evaluated by citric acid addition to acidify the GA uptake solution. In fact the reactivity of bioactive glasses will induce a basification of the functionalization medium, as discussed in the results and discussion section. 0.5 M citric acid (CA, Citric Acid Monohydrate, ACS reagent 99.0–102.0%, Sigma Aldrich) in double distilled water was added drop wise to the GA uptake solution till the pH was 3.0. A part of samples was functionalized with CA-modified GA solutions.

After 24 h, the GA and GA–CA solutions from each bottle were moved to clean bottles for UV analyses. The samples (powders and bulks) were washed twice by double distilled water, dried in an incubator for 12 h at 37 °C and stored in the dark. GA-grafted samples are labeled as SCNA + GA and CEL2 + GA, respectively, while